SZENT ISTVÁN UNIVERSITY
POSTGRADUATE SCHOOL OF VETERINARY SCIENCE

NEW DATA TO THE PATHOPHYSIOLOGY, CLINICS AND THERAPY OF BOVINE MASTITIS

PhD DISSERTATION

SZILÁRD JÁNOSI

CENTRAL VETERINARY INSTITUTE
BUDAPEST, HUNGARY

BUDAPEST
2002

Head of the Postgraduate School of Veterinary Science:
Professor Péter Rudas, DSc

Supervisor:
Professor Gyula Huszenicza, PhD

Department of Obstetrics and Reproduction,
Faculty of Veterinary Science, Szent István University
Budapest, Hungary

TABLE OF CONTENTS

1.1. LIST OF ABBREVIATIONS

5'D = 5'-deiodinase (e.g. activating enzyme of outer-ring deiodination producing T_3)
5D = 5-deiodinase (e.g. inactivating enzyme of inner-ring deiodination producing rT_3)
6-keto-$PGF_{2\alpha}$ = 6-keto-prostaglandin $F_{2\alpha}$
AcAc = acetoacetic acid
ACTH = adrenocorticotrop hormone
AM = ante meridiem
ANOVA = one-way analysis of variance
APE = acute putrid (endo)metritis (syn. = toxic metritis)
AST = aspartate aminotransferase
BHB = βOH-butyrate
BP = binding protein
C20:4 = arachidonic acid
C5a = complement 5a
CFU = colony forming unit
CL = corpus luteum
CMT = California Mastitis Test
CNS = coagulase-negative Staphylococci
COX-2 = cyclooxygenase-2
CRH = corticotrop releasing hormone
d = day(s)
DNA = dezoxy-ribonucleic acid
E.coli = Escherichia coli
GH = growth hormone (syn. somatotrop hormone, STH)
GN = Gram-negative
GP = Gram-positive
H.-E.stain = haematoxilin-eozin stain
h = hour(s)
HE = human equivalent
HPA = hypothalamic-pituitary axis
IC = intracisternal
IGFBP = IGF-I binding proteins
IGF-I = insulin-like growth factor-I (syn. somatomedin C)
IGF-II = insulin-like growth factor-II
IL-1, IL-2, IL6 = interleukin-1, 2 and 6
IM = intramuscular
IMI = intramammary infection
IU = international unit
LH = luteinizing hormone
LPS = lipopolysaccharide
LSCCM = low somatic cell count milk;
LSD = least significant difference
MIC = minimal inhibitory concentration
min. = minute(s)
mRNA = messenger ribo-nucleic acid
NDP mastitis = mastitis caused by **n**on **d**etected **p**athogens
NEB = negative energy balance
NEFA = non-esterified fatty acids

P. zopfii = Prototheca zopfii
P_4 = progesterone
PAS = periodic acid Schiff
$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$
PGFM = 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$-
PGI_2 = prostaglandin I_2
PMN = polymorphonuclear neutrophilic granulocytes
PRL = prolactin
Ps. aeruginosa = Pseudomonas aeruginosa
rT_3 = 3,3',5'-triiodothyronine (syn. = reverse-triiodothyronine)
S. aureus = Staphylococcus aureus
SCC = somatic cell count
SDS-PAGE = sodium dodecyl sulfate -polyacrylamide gel electrophoresis
Strep. = Streptococcus
T_3 = 3,3',5-triiodo-thyronine
T_4 = thyroxin
TCh = total cholesterol
TG = trigliceride
TNFα = tumor necrosis factor-α (syn. = cachexin)
TRH = thyreotrop releasing hormone
TSH = thyrotropin
TxA_2 = thromboxane A_2
TxB_2 = thromboxane B_2

2.1. SUMMARY

In Chapter 1 metabolic and endocrine aspects of bovine mastitis in early weeks of lactation are discussed. After reviewing the endocrine and reproductive consequences of certain endotoxin-mediated diseases, the results of the experimental studies are made known.

Due to the rapid genetic improvement nowadays many of the high-yielding cows suffer from clinical consequences (hepatic lipidosis and/or ketosis) of decompensated negative energy balance (NEB) in the postpartum period. In the early weeks of lactation these two metabolic complications often coincide, and are accompanied frequently by a high incidence of infectious diseases like acute (endo)metritis and mastitis, mostly due to a peri-parturient decrease in capacity of antimicrobial self-defense mechanisms. Up to now, however, we have not known yet whether the degree of this metabolic predisposition for mastitis is uniform regardless of the causative microbe. The pathogenesis of coliform mastitis is based on the host response to endotoxin. During this process leukocytes release several inflammatory mediators (tumor necrosis factor, interleukines etc.). These mediators can cause both severe inflammatory symptoms in the udder and disorders in the metabolic and endocrine functions of the cow.

The possible metabolic and endocrine predisposition for clinical mastitis outbreak as well as the mastitis related metabolic and endocrine changes were studied in 4 trials on large scale dairy herds.

The cows affected by mastitis in the puerperium had shown more elevated AcAc, BHB, NEFA and rT_3, and lower IGF-I, T_4 and T_3 levels previously than those remained healthy during the first 4 weeks after calving (*Exp. 1, 2*.). This tendency related to a more severe form of energy imbalance and derived mainly from parameters of mastitic cows infected with GP and GN environmental pathogens or affected by NDP mastitis. However, just after calving the data of those with *S. aureus* IMI were very close to their healthy herdmates. Significant predictive value was attributed only to the elevation of BHB, but not to any others of NEB related changes in circulating levels of hormones and metabolites. This predictive value was highly significant for GN microbes. Based on these findings we suppose that in the early weeks of lactation rather the hyperketonaemia than the NEB itself can predispose the cow for mastitis.

Elevated BHB levels were detected in the first samples of cows with NDP+GN mastitis taken within some hours after the outbreak of clinical symptoms. The BHB dependent character of NDP+GN mastitis was obvious in the first four weeks after calving. During the sampling period the BHB levels started to decrease and reached the physiological range within some hours.

Contrary to this continuously decreasing tendency in BHB, in the first some hours of the course a temporary elevation of NEFA level was seen in cows with NDP+GN mastitis, but not in those with GP mastitis. The observed NEFA increase might be the catabolic consequence of endotoxin induced endocrine changes.

About 23 % of the cows in *Exp. 2* showed < 40.00 nmol/l (e.g. lower than the mean - SD of symptomless, normoketonaemic cows; n=199) cortisol response to the ACTH challenge and were considered as *temporary hypocorticoid*. At the time of challenge all the cows were healthy, and neither the baseline level of cortisol, nor the degree of ACTH-induced cortisol response predisposed the cow for mastitis. However, if *hypocorticoid* cows were affected by GN or NDP mastitis in the first 14 days after calving, they showed more severe clinical symptoms and had higher risk for a fatal course, than their normocorticoid counterparts.

The LPS-induced cortisol increase was also detected in the *Exp. 1, 3* and *4* of the current trial. However, in our cases: in the 3 cows died in the earliest few days after calving in *Exp. 3*. no cortisol increase was detected at all. The cows in *Exp. 4* showed significantly lower ACTH-induced cortisol response in the early puerperium, than in the later stages of lactation. The two cows died of *E. coli* and NDP mastitis could hardly response to the ACTH challenge.

The clinical outbreak of mastitis was diagnosed in both of them also in the early puerperium. In cows with NDP+GN mastitis in the early puerperal phase the ACTH challenged cortisol increment inversely related to the severity of clinical symptoms.
These experiences have confirmed the regulatory role of physiological cortisol response in production and release of certain interleukins and TNFα in GN mastitis, emphasizing the clinical importance of temporary hypocorticism in postpartum dairy cows and the clinical importance of anti-inflammatory therapy in mastitic cows showing severe general symptoms of this disease.
Simultaneously with the LPS-induced cortisol elevation also a slight, temporary *progesterone* (P_4) increase was seen in cows with NDP+GN mastitis. This temporary P_4 increment was detectable only when no active corpus luteum was present on the ovary, and it was considered to be of adrenal origin as a side product of the LPS-forced cortisol synthesis.
Studying the thyroid gland function significant decrease in plasma levels of both the T_4 and T_3 (in *Exp. 3*), and diminished TRH-challenged T_4 and T_3 increase (in *Exp. 4*) were deteced, probably due to the cytokine loading must have been the most intensive in the severe forms of NDP+GN mastitis. These mastitis related changes were more obvious in more advanced stages of the course (in *Exp. 3*), were more pronounced in the early puerperium than in the late puerperium or during peak lactation (both in *Exp. 3* and *4*), and were extremely dramatic in the few cases died of mastitis soon after sampling. Contrary to the experiences in cows with NDP+GN mastitis, only mild or no mastitis-induced alterations were detected in animals affected by GP mastitis. The cows with NDP+GN mastitis were characterized by significantly more elevated rT_3 levels. These –supposedly endotoxin mediated – differences derived from the data of the most severe cases. This observation reveals that due to a mastitis related endotoxin loading in cows not only the 5'D-dependent activation of T_4 to T_3 may be impaired but also the capacity of its 5D-katalyzed inactivating pathway to rT3 can be increased: this may be a significant contribution for the LPS-induced decrease of T_4 in plasma.
The low prepartum T_4 levels in the cows which later exhibited GN mastitis may indicate either that cows with low T_4 status were more susceptible to infection by GN organisms or that the endotoxin-released products were already acting on the thyroid gland before clinical mastitis was evident. The immunosuppressive consequences of decompensated NEB (associated with low T_4 status), rather than the low plasma levels of T_4 and T_3 by themselves were supposed to predispose the cows for mastitis in *Exp. 1* and *2*.
The insulin and IGF-I levels of our cows in *Exp. 2* reflected the energy plus protein (but mainly energy) balance, and the low day 1-3 level of IGF-I in cows showing mastitis some days later confirm only the higher susceptibility of these individuals to intramammary infection caused by environmental pathogens in the early weeks of lactation. Significant independent associations of serum T_4 with T_3 and IGF-I levels during the puerperium were found in the *Exp. 1* and *2.*
A temporary elevation in plasma insulin was usually observed also in the first 2-3 samples of our cases with NDP+GN mastitis in *Exp. 3*. In complete agreement with the findings of model studies the IGF-I level was still almost unaffected at the time of this insulin elevation, and started to decrease continuously thereafter. Similar changes in insulin and IGF-I were seen almost never in cows with GP mastitis. These results have confirmed that the endotoxin-induced changes of both the GH-IGF-I axis and insulin participate in the shift of the metabolism towards catabolic events also in ruminants including the postpartum dairy cows.
Based on these results, we conclude that GN- (endotoxin-) mastitis related alterations in metabolic and endocrine systems may have practical importance in postpartum dairy cows. So it may be justified to exclude the mastitic cows (or at least of those with sever general symptoms) from trials studying the endocrine, metabolic and reproductive effects of certain feeding technologies and/or treatment procedures.

In Chapter 2 the drying off therapy of the bovine mastitis discussed. Antibiotic treatment at drying off aims at both eliminating the existing IMI and preventing new infections. During the dry period, elimination of the infection with antibiotics is more likely than during lactation as the drug is not milked out, and a higher and more uniform concentration of antibiotics is maintained in the udder. In addition, there are no economic losses due to discarding of milk containing antibiotic. Systemic dry cow therapy may have advantages: better distribution of the suitable drug in the udder tissue which may lead better cure of IMI. After reviewing the dry cow therapy, the results of the experimental study are made known.

Systemic (intramuscular, IM) vs. local (intracisternal, IC) routes of spiramycin-based drying off therapy were compared for efficacy on 65 *Staphylococcus aureus* infected udder quarters of 38 dairy cows. Single-dose (30 000 IU/kg) IM treatment (*single IM group*) resulted in a similarly low bacteriological recovery rate (14%) as seen in the untreated controls (18%). IM treatment (30 000 IU/kg) on 4 consecutive days (*4 IM group*) resulted in significantly higher quarter-based recovery rates than that in the *single IM group*. The bacteriological recovery rates obtained in the *intracisternal* and *4 IM groups* were quite similar but remained below 50%. Based on these findings as well as on the high costs of repeated intramuscular treatment regime there is no reason to give extra preference to the systemic application of spiramycin at drying off in the practice.

In Chapter 3 the bovine mastitis caused by *P. zopfii* alga is discussed. This form of mastitis have not been reported from Hungary previously. The reviewed disease attacks the high producing, machine milked dairy cows. It occurs worldwide in tropical and temperate climatic areas, and mostly appears sporadically in a therapy-resistant form. However, in poorly managed dairy herds it may be endemic, causing serious economic losses as a result of decreased milk quality and quantity and culling of infected animals.

In the years 1998-99 223 in 32 large-scale dairy herds cases of bovine mastitis by *Prototheca zopfii* were identified by the author. In the following years algae were isolated from several cows in more than 50 dairy herds. The ratio of algal mastitis increased from 2 % to 4.5 % in years 1999 and 2001, respectively. All of these farms were in Hungary, at a continental type, temperate zone climate. Both the sporadic and epidemic forms of *P. zopfii* mastitis were observed. Three of these dairy herds with a high incidence of algal mastitis were closely studied (*farm survey).* All the herds affected by the epidemic form had poor hygienic conditions and suffered from several managerial faults, but no specific predisposing factors could be identified. In most of the cases the *type II* variant of this pathogen was isolated. However, from 3 cows *type III* variant of *P. zopfii* was also isolated. This variant has not been isolated previously from bovine mastitis cases.

The cows had a higher chance of new infection in the early weeks of lactation and in the summer. The *P. zopfii* infection usually resulted in a chronic subclinical, or a mild clinical mastitis followed by a dramatic loss in milk production and permanent increase in somatic cell count. The histopathological findings could be characterized, as a progressive interstitial mastitis associated with alveolar atrophy. The self-recovery rate was very low.

Prototheca zopfii is concluded as a common mastitis pathogen of the dairy cows in Hungary as well.

2.2. ÖSSZEFOGLALÁS

Az értekezés **első fejezetében** a szerző a tejtermelő teheneknek az *ellés körüli időszakban előforduló tőgygyulladásaiva*l, különösen a mastitisnek az *anyagcserével és a hormonális rendszerrel összefüggő* vonatkozásaival foglalkozik. A nemzetközi szakirodalom alapján áttekinti az endotoxin-mediálta betegségek kórélettanát és endokrín következményeit, majd beszámol az elvégzett kísérletekről.

A nagy tejtermelésű teheneknél az ellést követő időszakban gyakran alakul ki a negatív energia-egyensúly (negative energy balance, NEB) következményeként májelzsírosodás és/vagy ketózis. A laktáció első heteiben, e két metabolikus zavarral együtt gyakran jelentkeznek bizonyos fertőző betegségek, mint a heveny endometritis és a klinikai tőgygyulladás.Ezek kialakulásában többnyire a szervezet antimikrobiális védekezőrendszerének ellés körüli, átmeneti működési elégtelensége játsza a fő szerepet.

Jelenleg nem ismert, hogy az említett metabolikus zavarok milyen mértékben hajlamosítják a tehenet a különböző kórokozók által előidézett tőgygyulladásra. A coliform baktériumok okozta mastitis kórfejlődésének alapja a szervezetnek az endotoxinokra (lypopoliszacharid, LPS) adott válasza. Ekkor a fehérvérsejtekből különféle mediátorok szabadulnak fel (pl. tumor necrosis faktor {TNFα}, interleukinek stb.), amelyek részt vesznek a mastitis kialakításában, de metabolikus és a hormonális rendszert érintő hatásaik is lehetnek.

A szerző négy -nagyüzemi méretű tejtermelő tehenészetben végzett- kísérletben vizsgálta a klinikai mastitisre hajlamosító, valamint a tőgygyulladást kísérő anyagcsere és hormonális zavarokat.

Az 1. és 2. kísérletben, a puerperium időszakában, tőgygyulladásban megbetegedett teheneknek, a betegség kialakulása előtt gyűjtött vérplazma mintáiban magasabb acetecetsav (AcAc), β-OH-vajsav (BHB), nem észterifikált zsírsav (NEFA) és reverz-trijódtironin ($rT_{3)}$, valamint alacsonyabb inzulinszerű növekedési faktor (IGF-I), tiroxin (T_4)és trijódtironin (T_3)szintek voltak mérhetők, mint az egészséges istállótársak mintáiban. Ez a tendencia arányos volt a NEB súlyosságával, és elsősorban a Gram pozitív (GP) és Gram negatív (GN) környezeti kórokozók, valamint a nem detektált patogének (NDP) által okozott mastitises tehenek adataiból származott. A *S. aureus* mastitises tehenek az egészségesekhez hasonló metabolikus és hormonális paraméterekkel rendelkeztek. A tőgygyulladás kialakulásának esélyét szignifikánsan (vizsgált paraméterek közül) a BHB szint jelezte előre. Ez a mastitis hajlamosságot jelző *korreláció különösen magasnak bizonyult GN baktériumok* esetében. Ezen eredmények alapján úgy tűnik, hogy a tehenek tőgygyulladással szembeni *fogékonysága* nem közvetlenül a negatív energia mérleg, hanem *elsősorban a* kialakuló *hyperketonaemia következménye*.

A NDP és GN tőgygyulladás klinikai tüneteinek megjelenését követő néhány órán belül vett, első mintákban emelkedett BHB szintet mértünk. Ez a jelenség a laktáció első négy hetében volt megfigyelhető. A mintavétel további részében a BHB szintje folyamatosan csökkent, és órákon belül elérte a fiziológiás értéket. A BHB szintjének folyamatos csökkenésével szemben, a NEFA szintjében, a klinikai mastitis megjelenését követően, egy átmeneti emelkedés volt mérhető, ami valószínűleg, az *endotoxin által kiváltott endokrín folyamatok katabolikus következménye*. GP mastitisben megbetegedett tehenekben ez a változás nem volt észlelhető.

A 2. kísérletben az ACTH-stimulációt követően a tehenek 23 %-a 40 00 nmol/l-nél alacsonyabb kortizolszinttel válaszolt, és így megfelelt az ún. *időleges hypocorticismus* ismérveinek. Az ACTH-stimuláció időpontjában valamennyi állat egészséges volt, és sem a bazális kortizolszint, sem az ACTH-stimulációra adott válasz mértéke nem volt szoros korrelációban a mastitis kialakulásával. Azonban, ha az ellést követő 14 napon belül egy ilyen hypocorticoid tehénnél GN vagy NDP mastitis lépett föl, a betegség klinikai tünetei szignifikánsan *súlyosabbak* voltak, és gyakoribb volt az *elhullással járó* végkifejlet, mint a normocorticoid istállótársak esetében.

Az LPS-indukálta kortizolszint-emelkedést az 1., 3. és 4. kísérletben is detektálni lehetett. Azonban a 3. és 4. kísérletben, a korai puerperiumban –GN és NDP mastitis miatt- elhullott

teheneknél nem vagy alig volt észlelhető a kortizolszint emelkedése, a betegség megjelenése után. A 4. kísérletben a korai puerperiumban az ACTH-stimulációra adott válasz szignifikánsan alacsonyabb volt, mint a laktáció későbbi szakaszaiban. Az ebben az időszakban előforduló GN és NDP tőgygyulladás súlyossága fordított arányban volt az ACTH-stimulációra adott válaszkészséggel.
A fent felsorolt eredmények megerősítik, hogy *GN mastitisben a kortizolnak fontos élettani szabályozó szerepe* van egyes interleukinek és a TNFα termelődésében és felszabadulásában, egyben aláhúzzák az ellés utáni időszakban megfigyelt *átmeneti hypocorticismus és a gyulladáscsökkentő terápia jelentőségét* súlyos klinikai tünetekkel járó tőgygyulladásban.
Az endotoxinok által indukált kortizolszint-növekedéssel egyidőben, átmeneti, kismértékű progeszteronszint- (P_4-) emelkedés is megfigyelhető volt GN és NDP mastitisben. Ezt az időleges P_4 emelkedést csak akkor lehetett észlelni, ha a petefészken nem volt aktív sárgatest. A mellékvesekéregben folyó intenzívebb kortizol-szintézis melléktermékének tekinthető.
A pajzsmirigy működését vizsgálva megállapítást nyert, hogy a *súlyos GN mastitis* során bekövetkező intenzív citokin-felszabadulás eredményeképp, szignifikánsan *alacsonyabb plazma T_4 és T_3 szint* (3. kísérlet), valamint *csökkent* mértékű *TRH-stimulációra adott T_4 és T_3 válaszkészség* (4. kísérlet) mérhető. Ezek az elváltozások erőteljesebbé váltak a betegség előrehaladásával (3. kísérlet) és fokozottan érvényesültek a korai puerperiumban (3. és 4. kísérlet). Különösen drámai változások voltak mérhetők a néhány mastitisben elhullott állat esetében. GP tőgygyulladás esetében hasonló folyamatok nem vagy csak alig észlelhető mértékben voltak megfigyelhetők. A GN és NDP mastitisben súlyosan megbetegedett teheneket szignifikánsan emelkedett rT_3 szint jellemezte. Ez a megfigyelés nemcsak arra utal, hogy endotoxin hatására tehenekben lecsökken a T_4 és a T_3 5'-dejodináz függő aktiválása, hanem arra is, hogy fokozódik ezen hormonok 5-dejodináz által katalizált inaktiválása (rT_3-má) is: ez jelentős tényező lehet az LPS-indukálta plazma T_4 szint csökkenésben.
A később GN mastitisben megbetegedett teheneknél az ellés előtt alacsonyabb T_4 szintet mértünk. Valószínűnek látszik, hogy ez is a dekompenzált negatív energia-egyensúly egyik következménye (1. és 2. kísérlet).
Az inzulin és IGF-I szintje a 2. kísérletben, tükrözte a tehén energia és fehérje egyensúlyát. A később mastitisben megbetegő teheneknél az ellés utáni 1-3. napon észlelhető, alacsonyabb IGF-I szint, megerősíti, hogy ezen állatok, a laktáció első heteiben fokozottan érzékenyek a környezeti kórokozók által előidézett tőgyfertőzésekre. Az 1. és 2. kísérletben szignifikáns korreláció volt a szérum T_4 és T_3 valamint IGF-I szintje között.
GN és NDP mastitisben megbetegedett tehenekben az első 2-3 mintában rendszerint emelkedett plazma inzulinszint volt mérhető (3. kísérlet). A modellkísérletek eredményeivel összhangban ebben az időpontban az IGF-I szintje még nem változott, annak csökkenése csak később kezdődött. GP tőgygyulladás során hasonló változásokat sohasem lehetett észlelni. Ezek az eredmények megerősítik, hogy az *endotoxin hatást követő katabolikus folyamatok megindításában mind a növekedési hormon-IGF-I tengely, mind pedig az inzulin részt vesz, ellés után lévő tejelő tehenekben is.*
Összegezve a felsorolt eredményeket megállapítható, hogy a GN (endotoxin) mastitishez kapcsolódó *metabolikus és endokrin változásoknak* ellés után lévő, tejtermelő tehenekben *gyakorlati jelentőségük van*. Ennek alapján javasolható, hogy a tőgygyulladásos teheneket (de legalább a súlyos tüneteket mutató állatokat) ki kell zárni az olyan kísérletekből, amelyek egyes takarmányozási és gyógykezelési technológiák metabolikus, endokrinológiai és szaporodásbiológiai hatását vizsgálják.

A **második fejezetben** a szerző áttekinti a tejtermelő tehenek ún. *szárazraállítási terápiájával* kapcsolatos irodalmi adatokat, majd beszámol a spiramycinnel végzett terápiás kísérleteinek eredményéről.
Az elapasztáskor végzett antibiotikumos gyógykezelésnek a célja kettős: kiterjed a a laktáció alatt fennálló tőgygyulladás gyógyítására és az elapasztás után várható fertőzések

megelőzésére. A szárazonállás alatt a fertőzések eliminálására nagyobb esély kínálkozik, mint a laktációban, mivel a beadott gyógyszert nem fejik ki, és a lecsökkent tejtermelés miatt magasabb és egyenletesebb hatóanyag-koncentráció jöhet létre. A laktáció alatt végzett gyógykezelések után az élelmezésügyi várakozási idő leteltéig a tejet a közfogyasztástól el kell vonni. Ez a jelentős gazdasági veszteség az elapasztáskor végzett gyógykezelést nem terheli. A megszokott intracisternális tőgyinfúzókkal végzett terápiával szemben a szisztémás (intramusculáris, intravénás) gyógykezelésnek várható előnye, hogy a megfelelő hatóanyag jobban eloszlik a tőgyszövetben, és ez nagyobb arányú bakteriológiai gyógyulást eredményezhet.
A szerző *intramuscularisan* (IM) és *intracysternalisan* (IC) alkalmazott, *spiramycin alapú szárazraállítási terápia hatékonyságát hasonlította össze* 38 tehén 65 *S. aureus* mastitises tőgynegyedében. Az egyszer alkalmazott IM kezelés (30 000 NE/ttkg) a kezeletlen kontrollhoz hasonló bakteriológiai gyógyulást eredményezett (14%<>18%). A 4 egymást követő napon ismételt IM terápia szignifikánsan jobb eredményt hozott (48%) (tőgynegyedenként végzett értékeléskor), hasonlóan az IC kezeléshez (40%). Ezek az adatok közel állnak a más gyógykezelési protokollal elért eredményekhez. A költség-hatékonyság számítás alapján a vizsgált gyógykezelési eljárások nem jelentenek áttörést a nehezen kezelhető mastitisek elleni védekezésben.

A **harmadik fejezetben** a szerző áttekinti a *Prototheca zopfii* alga által okozott tőgygyulladás nemzetközi szakirodalmát, beszámol a betegség első hazai megállapításáról, és az ezzel kapcsolatos kutatási eredményekről.
Az *algás tőgygyulladás* elsősorban a nagy tejtermelésű, fejőgéppel fejt szarvasmarhák betegsége. Világszerte előfordul, elsősorban trópusi-szubtrópusi éghajlat mellett, de a mérsékelt égövön is leírták. Általában sporadikusan jelentkezik, és terápia-rezisztens mastitisként kórjelzik, azonban nem megfelelő üzemi technológia/higiénia esetén halmozottan is előfordulhat. Ilyen esetekben a jelentős tejtermelés csökkenés, a tej magas szomatikus sejtszáma, és az indokolt állatselejtezések révén komoly gazdasági veszteségeket okoz.
A szerző 1998-1999-ben 32 tehenészet 223 tőgygyulladásos tehenéből mutatta ki a kórokozót. Az ezt követő években rendszeresen diagnosztizált *P. zopfii* mastitist, több mint 50 üzemben. 1999-ről 2001-re, 2%-ról 4,5%-ra emelkedett az algával fertőzött tehenek aránya a szerző által vizsgált tejmintákban. A betegség általában szórványosan, de közel 10 telepen, halmozottan fordult elő. 3 ilyen üzemben további vizsgálatokat is végzett a kórkép állományon belüli elterjedtségének, kórtani és klinikai jellegzetességeinek, illetve a fertőzés forrásának a tisztázására. Ezen farmok mindegyikén több, tőgygyulladásra hajlamosító technológiai hiányosságot talált, de specifikusan az algás tőgygyulladásért felelős tényezőt nem sikerült azonosítani. A legtöbb esetben a *P. zopfii* II. típusát tenyésztette ki a mastises tejmintákból, de három esetben a *P. zopfii* III. típusa bizonyult kórokozónak. Ezidáig ennek a típusnak a kórtani szerepéről a nemzetközi szakirodalomban nem jelent meg leírás.
Tapasztalatai szerint a termelés csökkenésével és a tej szomatikus sejtszámának (SCC) a hosszan tartó, jelentős fokú emelkedésével járó elváltozás többnyire idült lefolyású, tünetszegény, vagy szubklinikai, kórszövettanilag progrediáló, a mirigyvégkamra sorvadásával járó, interstitalis mastitisként jellemezhető. Az öngyógyulási hajlam nagyon gyenge, ezért a betegség elleni védekezés fontos eszköze a fertőzött állatok selejtezése.
A szerző adatai alapján a *P. zopfii* által okozott tőgygyulladással napjainkban az ország egész területén számolni kell.

3.1. GENERAL INTRODUCTION

Bovine mastitis can be defined as the response of the udder against various stimuli, particularly invading microbes. Acute mastitis is characterized by the classical signs of inflammation, such as swelling, pain, tenderness of the udder, fever and loss of function. The cow often fails to clear up the infection: mastitis pathogen bacteria persist for months within the udder and are shed in the milk. Chronic inflammation results in decreased milk production with increased milk somatic cell count.

Bovine mastitis has a great impact on the economy of dairy industry. The disease causes decrease in milk production and in milk quality resulting in severe losses for both the producers and processors of milk. An additional loss is represented by the cost of antimicrobial therapy and the discarded milk contaminated with drugs.

As a result of the changes of feeding, housing and milking systems and by the development of different mastitis control programs the epidemiology of this disease has dramatically changed. This process is noticeable also in Hungary.

Dairy farming has changed dramatically in the last century. In 19th century the average number of cows per farm was below five and the animals grazed in fields in the summer, and they were kept in small barns in winter on soil straw litter. The cows were fed mainly dry hay and milked by hand. The average annual milk production was about 2000 kg per cow. The prevalence of mastitis at that time is not known, but seemingly it was a minor problem that time. Since the beginning of the 20th century a concentration of farmed cows started in Europe and also in Hungary. This process was associated with more intense feeding and keeping which led to higher milk production but unfortunately it came with a higher risk of production diseases including mastitis too. Several studies demonstrated that high-producing cows are at increased risk of infectious diseases (Destilleux et al., 1994; Gröhn et al., 1989 and 1994; Oltanecu and Ekesbo, 1994; Uribe et al., 1995).

The mastitis caused by Strep. agalactiae was the first case of mastitis resulting in high economic losses in Hungarian dairy farms (Hetzel, 1929).

After introducing antibiotics into the mastitis therapy (1945), most of the highly susceptible Strep. agalactiae strains had been replaced by bacteria such as *S. aureus* which are more resistant to the antibiotic therapy in the udder. At present time Staph aureus is the most frequent contagious mastitis-pathogen germ in Hungary (unpublished data of the Central Veterinary Institute, Budapest). The milking machine is the main source of new animal to animal intramammary infections by *S. aureus*. In addition, the improper machine milking is an important factor to support bacteria to colonize teat ends and reduce the capacity of self defense mechanism of the udder (Pyörälä, 1995a)

Despite of efforts of several researchers worldwide there is no antibiotic and treatment regime, which is able to eliminate *S. aureus* from the udder and reliably cure the chronic udder inflammation (Sandholm, 1995b).

In the past decade, the standard mastitis control program has suggested hygiene and management practices to control intramammary infection (Neave et al., 1969). A decrease in bulk milk somatic cell counts (SCC) is an indicator of the success of the control programme. Although farmers with low SCC herds were able to decrease the prevalence of mastitis with contagious pathogens, these herds still show a high incidence of clinical mastitis by environmental pathogens (Lam et al., 1997; Schukken et al., 1989). In several low SCC herds coliform bacteria (mainly E.coli) are major causes of clinical mastitis (Barkema et al., 1998; Schukken et al., 1989)

Low somatic cell count in milk seems to predispose to coliform mastitis. Somatic cells are parts of the defense mechanism of the udder. The udder becomes more sensitive to coliforms as the SCC decreases (Sandholm and Pyörälä, 1995a).

An increased incidence of clinical mastitis caused by environmental pathogens was reported to be directly associated with impairment of cow defence mechanisms mostly in the early postpartum period (Kremer et al., 1993b; Van Werven et al., 1999).
Transition from pregnancy to lactation involves considerable metabolic adaptations in all mammals especially in cows selected for a high rate of milk production due to the sudden demand for large quantities of glucose in an animal with a ruminal digestive system. Rapid adaptation requires immediate changes in the rates of synthesis-secretion and degradation-elimination of endocrine regulatory molecules which is reflected by characteristic changes in their circulating concentrations (Hoshino et al. 1991; Hydbring et al. 1999; Kunz and Blum 1985; Pethes et al. 1985; Schams et al. 1991; Skaar et al. 1991; Vega et al. 1991). However, the ability of a cow to adapt successfully may be compromised during episodes of postparturient mastitis and other infections, particularly those caused by Gram-negative pathogens, which release lipopolysaccharide (LPS) from the outer cell wall membrane during the inflammatory process.
The pathogenesis of coliform mastitis is based on the host response to endotoxin originated from the cell wall of bacteria. During this process several inflammatory mediators (tumor necrosis factor, interleukines etc.) release from leukocytes. These mediators can cause both severe inflammatory symptoms in the udder and disorders in the metabolic and endocrine functions of the cow (Kenison et al., 1991; Sandholm and Pyörälä, 1995a).
The present Hungarian dairy herds are on different stages of this evolution of mastitis epidemiology.
In my Ph.D. work I studied some special aspects of bovine mastitis having practical importance in Hungarian dairy herds.

3.2 AIMS OF THE STUDY WERE TO

1. Evaluate whether the predisposition for clinical mastitis resulted from contagious or environmental pathogens in the first some weeks of lactation is influenced by the immediate postpartum metabolic condition of the cow.
2. Evaluate the known periparturient tendencies in plasma levels of certain metabolic hormones influenced by mastitis in puerperium.
3. Evaluate whether the endocrine alterations known from model studies can be recognized in pathogenesis of clinical mastitis also in the practice
4. Evaluate whether the presence of temporary hypocorticism interferes with the course of this disease.
5. Evaluate the efficacy of intramuscular vs. intracisternal spiramycin dry cow therapy against *S. aureus* mastitis.
6. Evaluate the microbiology, pathology and epidemiology of the bovine mastitis caused by the alga Prototheca zopfii

4. CHAPTER 1

METABOLIC AND ENDOCRINE ASPECTS OF BOVINE MASTITIS IN EARLY WEEKS OF LACTATION

4.1. ENDOCRINE AND REPRODUCTIVE CONSEQUENCES OF CERTAIN ENDOTOXIN - MEDIATED DISEASES IN FARM MAMMALS. A REVIEW

4.1.1. PATHOLOGY OF ENDOTOXINS

The medical interest in endotoxins is as old as our century. The term *endotoxin* (synonyms: O, or somatic antigen) refers to the part of the cell wall of Gram negative bacteria which is composed of polysaccharides, phospholipids as well as of small amounts of protein called together as lipopolysaccharides (LPS). The endotoxin molecule consists of 3 main subunits: (a) the O-polysaccharide, imparting serospecificity for Gram negative bacteria, (b) the lipid moiety, which is considered to be the toxic component of the cell wall, and (c) the R core, consisting of hexoses, hexamines and heptose, which act as bridges between the other two components (*Fig 1*) (Sandholm and Pyörälä, 1995a; Westphal, 1975).

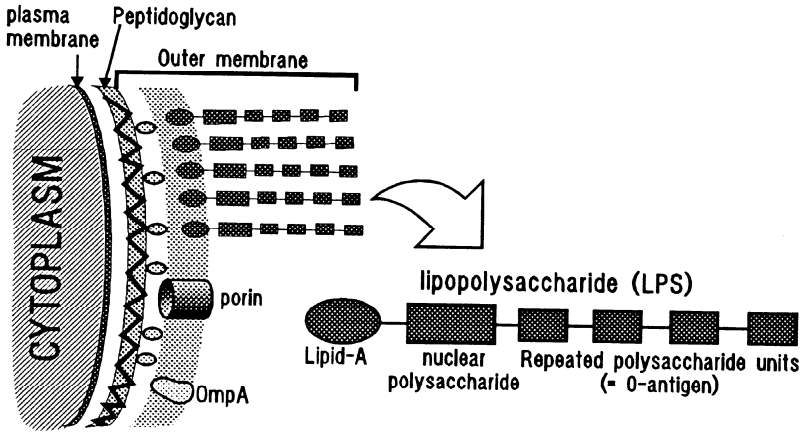


Fig 1.: The cell wall structure of Gram negative bacteria (after Sandholm and Pyörälä, 1995a)

Both of the laboratory and farm animals can be exposed to LPS from exogenous and endogenous sources: Endotoxins can be (a) exogenously *injected* with contaminated drugs or in experimental situation, (b) *liberated* in various disease (mastitis, laminitis, or septicaemia caused by Gram negative pathogens), and (c) *absorbed* from the postpartum uterus or – under certain circumstances – from the rumen and/or gastro-intestinal tract. Endotoxins are also present in the environment, stemming from the growth of Gram negative bacteria on plants and other sources of animal feed. Therefore, animals can be exposed to endotoxins not only from infection of Gram negative bacteria, but also – at least in pathological cases – by resorption from endotoxin contaminated fodder, and / or due to the Gram negative bacteria residing in the gastro-intestinal tract. In ruminants the rumen fluid contains large amounts of endotoxins which increase when animals are fed on concentrates instead of hay. Increased LPS absorption from forestomach has been demonstrated in complex disease syndromes such as ruminal acidosis, ruminal stasis and parturient paresis in the cow. In experimental models standard endotoxin producing (usually *Escherichia coli*) cultures, or LPS containing preparations may be administered by parenteral ways and also perorally (Holst and Kindahl, 1995; Jarlov et al., 1992; Yagoda et al., 1990). Of them the peroral / gastro-intestinal way of LPS loading seems to be the most problematic: Even if after peroral LPS administration slight to severe clinical symptoms and also biochemical consequences of endotoxicosis have been reported to occur in both of the monogastric animals (pig) and ruminants (goat) (Cort et al., 1990; Holst

and Kindahl, 1995), probably the portal vein endotoxemia (without bacteremia) of intestinal origin must be regarded as a physiological state. Endotoxins absorbed from the gastro-intestinal tract must be passed to the liver where they may be removed, mainly by the Kupffer cells (Fox et al., 1990). Bile acids are known to have determinate importance in the detoxification of endotoxins in the gastro-intestinal tract (Bertók,1983; Kocsár et al.,1960;). The clearance capacity of a normal liver has been shown to exceed the endotoxin amounts normally present in the portal blood in rats (Yamaguchi et al., 1982). The endotoxin load is known to increase in different gastro-intestinal disorders and thereby overload the hepatic detoxifying capacity, as in dogs with hemorrhagic enteritis (Wessels et al., 1987). Malnutrition is another condition that may lead to endotoxemia of gastro-intestinal origin: it has been shown that protein malnutrition with LPS containing nutrients may destroy the intestinal mucosal barrier (Deitch et al., 1987; Klein et al., 1988). In ruminants also the rumen can be the source of endotoxin-overload in certain cases (ruminal stasis induced by hypocalcaemia or certain drugs; ruminal acidosis) (Andersen et al., 1990, 1994b; Aiumlamai, 1991).The impairment of the liver function due to cirrhosis, hepatitis or hepatic lipidosis, may also result the permeation of endotoxins of gastro-intestinal origin to the systemic circulation (Ravin et al., 1960). The clearance ability of the liver is therefore an important factor in the prevention of the spill-over of endotoxins which has been confirmed also in ruminants (Andersen et al., 1996).

Although there are limited data available in the literature, it is generally accepted that absorbed or experimentally administered endotoxins disappear rapidly from the circulation. Clinically healthy cows cleared endotoxin from the peripheral plasma within 30 min. (Andersen et al., 1996) which may explain the limited success or failure of attempts to detect endotoxin in bovine systemic blood in Gram negative mastitis or in ruminal disorders (Aiumlamai, 1991; Andersen et al., 1994b; Katholm and Andersen, 1992). According to this phenomenon indirect hematological and biochemical consequences rather than the direct determination of LPS are used to confirm the diagnosis of endotoxicosis even under experimental conditions (Aiumlamai, 1991; Lohuis, 1989; Sandholm and Pyörälä, 1995a). As an effect of the non-steroid antiinflammatory substances on liver metabolism, the plasma disappearance time of experimentally injected endotoxin was 2-3 times longer in cows pretreated with flunixin meglumine, and 6-12 times longer in cows pretreated with phenylbutazone than in their healthy counterparts (Andersen et al., 1996). Even more marked effect may be attributed to the spontaneously developed periparturient hepatic lipidosis: none of the 4 cows treated with 25 μg standard (*Escherichia coli* 055:B5) endotoxin were able to clear the injected LPS dose within a 360 min. long sampling period and one of them died before the end of the study. These results support the theory that *cows suffering from hepatic lipidosis are more susceptible to endotoxins*, originating from the gastro-intestinal tract or from organs (udder, uterus) infected with Gram negative bacteria, than healthy cows. The often stated failure of therapeutic success in the cow suffering from fatty liver may be ascribed (at least partly) to these findings, especially when drugs depending on liver metabolism are used (Andersen et al., 1996). The phenomenon may have clinical importance in dairy cows affected by Gram negative mastitis in the early postpartum period.

The pathophysiological consequences of endotoxin exposure have an obvious dose-dependent character, being more severe if (a) the *endotoxin neutralizing capacity* of the *organ* (*rumen*: in case of acidosis; *udder*: in the colostral period) or of the *whole organism* (for instance when the bile acid secretion is impaired due to simultaneous liver damage) is depressed, or (b) the *resorption of endotoxins* has been shown to increase (ruminal stasis, postpartum uterus, mastitis or laminitis caused by Gram negative bacteria). Generally, as the dosage of endotoxins increases, the latency time decreases, the peak effect becomes more pronounced and the duration of the effect protracted. Furthermore, individual susceptibility, age, pregnancy, charge of

the endotoxin and the route of exposure / administration influence significantly the degree of distress(Andersen et al., 1990, 1994a and 1994b; Cullor, 1992; Giri et al., 1990; Jackson et al., 1990; Jarlov et al., 1992; Lohuis, 1989; Peter et al., 1987 and 1990; Sandholm and Pyörälä, 1995a; Smith, 1986).

Recently it has been known, that endotoxins appear to have little direct toxic effects. The most severe clinical, hematological and blood chemical changes are indirect consequences, associated with a cascade of biological processes initiated by endotoxins (Dinarello, 1983; Sandholm and Pyörälä, 1995a; Smith, 1986). In vitro or in vivo exposure of neutrophyls, phagocytic cells and platelets to endotoxin results in synthesis and release of increased amounts of many biologically active substances, i.e. various protein-like *cytokines* (α-tumor necrosis factor, TNF_{α}, interferons, as well as several members of the interleukin family), *biogenic amines* (histamine, serotonine), *oxygen radicals* (nitric oxide, hydrogen peroxide, superoxide) and – through the activation of the cyclooxygenase-2 (COX-2) and lipoxygenase enzyme systems – certain arachidonic acid (C20:4) derived lipid-like molecules such as *prostanoids* (prostaglandins including prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$ and prostaglandin I_2, PGI_2, as well as thromboxanes including thromboxane A_2, TxA_2; *Table* 1) and *leukotrienes*. The adequate release of these mediator substances is beneficial for the organism (moderate fever, generalized stimulation of the immune system, killing of microbes), their imbalanced overproduction, however, may lead to deleterious, harmful consequences (general depression, high fever, respiratory distress, disturbance in the motility pattern of the gastrointestinal – and urogenital – tract, extreme polymorphonuclear leucopenia followed by leucocytosis, alterations in the carbohydrate, lipid and protein metabolism, redistribution of certain trace metals through the body, and in the most severe cases profuse diarrhea, low blood pressure, disseminated blood clotting and at the end lethal shock). Almost all of these harmful consequences may be induced by experimental parenteral (intravenous or intramammary) administration of endotoxins or TNFα alone (Hirvonen et al., 1999; Jarlov et al., 1992; Sandholm and Pyörälä, 1995a; Smith, 1986).

4.1.2. ENDOCRINE INTERACTIONS AND THEIR POSSIBLE REPRODUCTIVE CONSEQUENCES IN ENDOTOXIN MEDIATED DISEASES

Both in vivo release and experimental / accidental administration of endotoxins are followed by characteristic endocrine events and – at least under certain conditions – by metabolic and / or reproductive consequences.

The *temporary activation of the COX-2 enzyme system* and its consequences has been extensively studied. The temporary activation of the COX-2 by endotoxins may lead to increased production of prostanoids including $PGF_{2\alpha}$, and its stabile metabolite (15-keto-13,14-dihydro-$PGF_{2\alpha}$,, PGFM) which can cause luteolysis when $PGF_{2\alpha}$-sensitive corpus luteum (CL) is present on the ovary. This mechanism is capable of shortening of the estrous cycle with a premature return to estrus *in non-pregnant ruminants* and *Equidae* (but not in pig , due to the 13-day long $PGF_{2\alpha}$-insensibility of their developing CL after ovulation). Clinical importance can be attributed to this phenomenon first of all in postpartum dairy cows in which the activation of C20:4 cascade by endotoxins absorbed from the uterus and the subsequent increase in $PGF_{2\alpha}$ and PGFM levels (a) seem to inhibit the first ovulation and CL formation, and (b) may play a role in shortening the life-span of the first CL after calving. In endometritic cows and mares the latter phenomenon may occur also later in the postpartum period (Battaglia et al., 1999, 2000; Cort et al., 1990; Gilbert et al., 1990; Harris et al., 2000; Kindahl et al., 1992 and 1996; Peter and Bosu, 1987 and 1988; Peter et al., 1990; Stabenfeldt and Edqvist, 1990). Of greater importance is, however, what happens to *pregnant animals*. It is

well known that abortion occurs commonly following Gram negative bacterial infections in many species (in mice, pigs, mares and also in ruminants). However, the primary mechanism by which Gram negative bacteria are capable of inducing abortion is not clearly understood in all cases. If complete luteolysis occurs due to the endotoxin induced COX-2 activation and subsequent $PGF_{2\alpha}$ release, abortion will take place within 24-48 h. This abortifacient property of luteolysis can be completed by the oxytocin-like uterotonic character of $PGF_{2\alpha}$ (Garcia-Villar et al.; 1985). Abortion due to endotoxin induced $PGF_{2\alpha}$ release has been reported to occur in *goat* during the entire pregnancy and in the *sow* at least until day 80 of gestation (Cort and Kindahl, 1986 and 1990; Fredriksson et al., 1985), e.g. in species in which the progesterone production of the primary CL is responsible for the maintenance of pregnancy during the entire gestation (Stabenfeldt and Edqvist, 1990). In *cows* the endotoxin exposure is also followed by a marked, dose dependent increase in the plasma $PGF_{2\alpha}$ level with a subsequent drop in progesterone concentration (Giri et al., 1990). In cattle, however, the pregnancy is maintained by progesterone secreted exclusively by the CL only in the early stage of gestation. Later, in more advanced gestation the progesterone from extraluteal sources (mainly from placenta, but perhaps also from adrenal cortex of the dam) has physiological importance in the maintenance of pregnancy (Stabenfeldt and Edqvist, 1990). Based on this phenomenon, although endotoxins may lead to abortion also in cows in all stages of gestation, this effect is more considerable in the first trimester than in the second or third trimester of pregnancy (Giri et al., 1990). Altough, the *mare* is sensitive to endotoxin induced abortion only up to about day 50-60, e.g. by the time (a) of achievement in function of secondary CLs continuously forming in the ovary for about 2 months after day 35-40 of gestation and (b) of the onset of placental gestagen production (Daels et al., 1987 and 1991b; Kindahl et al., 1991). It should also be pointed out in various species that sometimes endotoxins can result in only a temporary fall in progesterone production without causing abortion (Cort and Kindahl, 1986 and 1990; Daels et al., 1987 and 1991b; Giri et al., 1990; Kindahl et al., 1991 and 1996). Otherwise in addition to the luteolytic and uterotonic effects of $PGF_{2\alpha}$ other C20:4 derivats such as TxA_2, a potent platelet aggregator and vasoconstrictor as well as PGI_2, a known vasodilatator causing hypotension may contribute to the pathophysiologial process resulting in abortion through their local effects directed to the feto-placental unit (Giri et al., 1990). In other cases the endotoxin exposition is known to cause bleeding and necrosis in placental tissues, and if this damage is excessive enough, secondary harm to the fetus will naturally result through non-endocrine way (Kindahl et al., 1996). The inhibition of COX-2 activity by flunixin meglumine (and also by other non-steroid antiinflammatory drugs) has been reported to counteract the $PGF_{2\alpha}$-mediated abortifacient effect of endotoxins in domestic mammals (Andersson et al., 1986; Cort and Kindahl, 1990; Daels et al., 1991a; Jarlov et al., 1992; Harris et al., 2000).

There are less data available in the literature on other endotoxin influenced endocrine mechanisms. It is known, that endotoxins resorbed into the circulation and/or the endotoxin-induced cytokines, mostly TNF_{α} will provoke (a) the hypothalamic/adeno-hypophyseal CRH / ACTH and TRH / TSH and prolactin release with a subsequent increase in thyroid hormone and adrenocortical glucocorticoid (in most of the domestic mammals: cortisol) production, (b) as well as a some day long decrease in insulin-like growth factor (IGF)-1 and IGF-binding protein-2 level, (c) but only a short reduction in the growth hormone (GH) blood concentrations (Briard et al., 2000; Bosu et al., 1995; Elenkov et al., 1992; Elsasser et al., 1995; Jackson et al., 1990; Sandholm, 1995b; Shuster and Harmon, 1992; Sordillo and Peel, 1992; Yu et al., 1998).

In laboratory animals and cattle this kind of increased *glucocorticoid* release can be stimulated by experimental endotoxin or TNF_{α} administration also directly and not only through the CRH / ACTH mediated pathway (Bosu et al., 1995; Elenkov et al., 1992), perhaps

due to the fact, that great pathophysological and clinical importance can be attributed to these hormones in the down-regulation of the local TNF_α production on the site of the inflammatory process. Under physiological conditions this feedback-like mechanism is thought to be responsible for the prevention of the harmful (sometimes life-threatening) overproduction of certain cytokines, mainly TNF_α. After a single intravenous or intramammary administration of LPS, or in a simple case of Gram negative mastitis in cattle with normal adrenocortical responsiveness, this plasma cortisol level increase starts already a few minutes after the beginning of the endoxin effect, it reaches a peak concentration after 3-4 hours and does not last longer (except for fatal cases) than 24 hours. By this time, however, the endotoxin stimulated cytokine increase including the plasma level of TNF_α drops again to basal level (Elsasser et al., 1995; Hardie and Krouse-Elliot, 1990; Jackson et al., 1990; Lohuis, 1989; Sandholm, 1995b; Shuster and Harmon, 1992; Sordillo and Peel, 1992). If a higher dose (2.5 μg/kg) of endotoxin was administered in a form of a 6 hour long intravenous infusion, elevated cortisol levels were detected for a longer (about 60 hours) period (Giri et al., 1990). The high cortisol level caused by endotoxins resorbed from the postpartum uterus has been reported to suppress the preovulatory LH surge and so it may be one of the factors resulting in delayed resumption of cyclicity and / or formation of anovulatory cysts in dairy cows affected by uterine complications some weeks after calving (Bosu and Peter, 1987; Peter et al., 1989). On the other hand the temporary hypocorticism is considered to be a quite frequent phenomenon in dairy cows in the first postpartum week, but actually there are only very few or no data are available in the literature on its effect on the course of endotoxin mediated diseases (Kolk, 1991; Thuroczi et al., 1996).

The endotoxin-induced TRH release has been reported to lead to a subsequent increase in thyroid hormone (thyroxine, T_4) and prolactin (PRL) production in cows (Jackson et al., 1990; Sandholm, 1995b). This change in T_4 level may be important in the thermoregulation and also in the regulation of the metabolic consequences of endotoxin mediated diseases (Sandholm, 1995b). In cows an obvious increase in PRL production was only observed when endotoxin was injected intravenously and not intramammarily (Jackson et al., 1990), and its role is supposed in the immunmodulation but not in the milk production of the udder. In sows, however, the experimental endotoxin administration decreased both PRL level and milk production in the first week after farrowing, but not later (Smith and Wagner, 1984 and 1985). In laboratory animals (rat) a high dose infusion of endotoxin was demonstrated to decrease both the peripheral level of thyroid hormons and the TSH-induced T_4 responsiveness through a membrane damaging effect of LPS. In a recent study on cows, Kahl et al. (2000) reported decreased 5'-deiodinase acivity in the liver, which resulted in lower T_3/T_4 ratio and a decreased plasma concentrations of thyroid hormones. Pre-treatment with radiodetoxified endotoxin was proved to be an effective method in prevention of this detrimental endocrine consequence (Bertók, 1998; Bertók and U. Nagy, 1984; U. Nagy et al., 1983; U. Nagy and Bertók, 1990;). Similar observation, however, has not yet been reported in farm animals.

The endotoxin induced alterations in IGF-1, IGF-binding protein-2 and GH levels – and presumably also in glucocorticoid and T_4 concentrations – may be responsible for the LPS related metabolic events shifting the metabolism from an anabolic to the catabolic state (Clemmonds and Underwood, 1992; Elsasser et al., 1994 and 1995; Kenison et al., 1991; Sandholm, 1995b; Sartin et al., 1998). Significant and prolonged decreases in plasma concentrations of IGF-1, which are highly correlated with the nitrogen status of animals, have been associated with the progression of disease stress (Elsasser et al., 1988 and 1989). In pregnant cows a 6 hour long intravenous endotoxin infusion at a dose of 1 or 2.5 μg/kg caused an initial hyperglycemia for 1-3 hours which was followed by a gradually developing hypoglycemia persisting as long as 18 and 30 hours in the low and high dose groups, respectively. In the high dose group elevated lactic acid levels were also found for about 48 hours. Endotoxin

infusion at both doses caused a preferential mobilization of oleic acid from adipose tissue, and also had some effects on the mobilization of palmitic and stearic acids during the post-infusion periods (Giri et al., 1990). Recombinant TNF treatments of dairy heifers induced a condition of hyperinsulinemia without hyperglycemia, indicating reduced insulin sensitivity (Kushibiki et al., 2000). In lambs a catabolic effect in protein metabolism was attributed to endotoxin induced TNF_{α} increase which might be prevented with IGF-1 pretreatment (Douglas et al., 1991). Although no data have been found in the literature to support this idea, the above mechanisms may have clinical relevance in the pathogenesis of secondary ketosis in postpartum dairy cows.

4.1.3. ENDOTOXIN-ASSOCIATED MASTITIS AS A POSSIBLE INFLUENCING FACTOR OF REPRODUCTIVE PERFORMANCE IN DAIRY COWS DURING THE EARLY LACTATION

In well managed dairy herds producing bulk milk with low (< 150 000 - 250 000) somatic cell count (SCC) in the first 3-4 postpartum weeks significantly higher rate of cows have been reported to suffer from acute mastitis than later in the lactation. The predominant part of these cases were caused by opportunistic bacteria including endotoxin containing Gram negative strains (E. coli, sometimes Klebsiella ssp. and others) (Barkema et al., 1998.; Green et al., 1996; Hogan et al., 1989; Miltenburg et al., 1996; Schukken et al., 1989). The risk for early postpartum mastitis can be coupled with retained fetal membrane and subsequent putrid endometritis (Schukken et al., 1988). In spite of the known pathophysiological principles (Anderson et al., 1986; Giri et al., 1984; Katholm and Andersen, 1992; Sandholm, 1995b; Sandholm and Pyörälä, 1995a) only a few data is available concerning the clinical and reproductive importance of the above-mentioned endocrine consequences of the endotoxin-associated (toxic) mastitis under practical (farm) conditions. Moore et al. (1991) reported, that in a herd where E. coli was the predominant mastitis pathogen, the cows were almost two times more likely to have an altered inter-estrus interval followed by an episode of clinical mastitis compared to herdmates without clinical mastitis. A similar alteration in inter-estrus interval was not observed in a Staphylococcus aureus affected herd. However further studies are required to confirm the supposed effect of Gram negative mastitis on ovarian function in which relevant endocrine methods should be used to follow up these consequences.

4.2. OWN STUDIES:

METABOLIC AND ENDOCRINE ASPECTS OF BOVINE MASTITIS IN EARLY WEEKS OF LACTATION

4.2.1. INTRODUCTION

Due to the rapid genetic improvement of nowadays many of the high-yielding cows suffer from clinical consequences (hepatic lipidosis and/or ketosis) of decompensated negative energy balance (NEB) in the postpartum period. In the early weeks of lactation these two metabolic complications often coincide, and are accompanied frequently by a high incidence of infectious diseases like acute putrid (endo)metritis (APE; syn.: toxic metritis) and mastitis (Correa et al., 1993; Erb and Gröhn, 1998; Markusfeld, 1985; Oltanecu and Ekesbo, 1994; Schukken et al., 1988; Valde et al., 1997), mostly due to a peri-parturient decrease in capacity of antimicrobial self-defense mechanisms. Impairments in immunophenotypical and functional properties of polymorphonuclear neutrophilic (PMN) granulocytes and monocytes induced by elevated level of ketone bodies, and other related metabolic changes (Cai et al., 1994; Klucinski et al., 1988a and 1988b; Kremer et al., 1993b; Sartorelli et al., 1999 and 2000; Suriyasathaporn et. al., 1999 and 2000; Zerbe et al., 2000) seem to be the key process of this phenomenon. Up to now, however, we do not know yet whether the degree of this metabolic predisposition for mastitis is uniform regardless of the causative microbe, or in this respect there may be differences between cases caused by contagious (*S. aureus*) or Gram-positive (GP) and Gram-negative (GN) environmental pathogens (e.g. in most of the cases *Strep. uberis*, *Strep. dysgalactiae*, other or fecal *Streptococci*, and *E. coli*, *Klebsiella* ssp., other *Enterobacteriacae*, *Ps. aeruginosa*, respectively)

The intensive release of cytokines [tumor necrosis factor-α (TNFα) and certain members of the interleukin family (IL-1, IL-2, IL-6)], as well as of eicosanoids, nitrous oxide and other mediators are the known consequences of an extended inflammatory process including the severe clinical form of bovine mastitis (Sandholm, 1995b; Sandholm and Pyörälä, 1995a). This release of various mediators is obvious mainly in cases caused by GN pathogens yielding significant quantity of *endotoxin* (syn. *lipopolysacharides*, LPS) from the cell wall of these bacteria (mainly of *E. coli*). The LPS related elevation in plasma levels of cytokines can increase the adrenocortical cortisol production, which is an important down-regulatory mechanism, thought to be capable of preventing the overproduction of TNFα and some other inflammatory mediators (Elsasser et al. 1995; Jackson et al. 1990; Sandholm and Pyoräla 1995a). The above normal cortisol response to a standard low-dose adrenocorticotrop hormone (ACTH) challenge has been reported to occur sometimes in postpartum dairy cows (Kolk, 1991; Kolk et al., 1991; Kulcsár et al., 1996; Torres et al., 1997b). We do not know, however, whether this phenomenon may interfere with the course of mastitis. Cytokine related changes have also been recognized also in circulating levels of 3,3',5-triiodo-thyronine (T_3), thyroxin (T_4), insulin, insulin-like growth factor-I (IGF-I) and IGF-I binding proteins (IGFBP) (Sandholm and Pyörälä, 1995a). These LPS / cytokine induced endocrine changes were observed, however, mainly in highly standardized model studies employing experimental (intravenous, intramammary) endotoxin or TNFα challenge, rather than in natural cases of mastitis in cows kept under commercial farm conditions.

The aims of this trial were to study whether (1) the known periparturient tendencies in circulating levels of certain metabolic hormones are influenced by a mastitis outbreak, (2) the predisposition for clinical mastitis resulted from contagious vs. environmental pathogens in the first some weeks of lactation is influenced by the immediate postpartum metabolic condition of the cow, (3) the endocrine alterations known from model studies can be recognized in pathogenesis of clinical mastitis also in the practice, (4) as well as the presence of temporary

hypocorticism may interfere with the course of this disease. For this purpose four series of experiments were conducted on group-fed high-yielding dairy cows kept in large-scale dairy herds.

4.2.2. MATERIALS AND METHODS

4.2.2.1. Farm conditions

The trial was carried out in 4 commercial large-scale dairy units (*Exp. 1:* in Herd A only; *Exp. 2*: in all the 4 herds; *Exp. 3* and *4*: in Herds A and D) with about 500 to 1850 Holstein-Friesian cows and their crosses in each, producing about 7000 - 8300 kg fat corrected milk per cow. Each farm had its own mastitis control program (Herd A represented one of the top farms of Hungary), and was free of *Strep. agalactiae.* Moreover, all recognized cases of *S. aureus* mastitis were immediately separated, although they were also culled soon only in Herd A. All 4 farms could produce low somatic cell count milk (LSCCM) for many years. In all the herds the cows were kept under free housing conditions in sheds connected with open air pens year round with no possibility for pasturing and individual feeding, in groups of 70 to 100 animals formed in accordance with their stage of lactation and their monthly checked actual daily milk yield. In the sheds straw-bedded resting place were provided for the animals. Dry cows were housed separately and they calved in maternity units in groups of 3 - 4 animals. The calves were removed from their mothers when the dams left the unit at the end of the colostral period. Separated groups were formed for fresh milking heifers and cows. The cows were milked in milking houses three times (all the fresh milkers in the first about 60 days, plus those producing >30 kg milk/day) or twice (yielding about <30 kg milk/day) a day in Herds A, C and D, but only twice in all the cases in Herd B. The milking parlors were furnished with BouMatic (Herd A, B and C), or AlfaLaval (Herd D) type machinery. The hygienic conditions were equal to the European standard. Their daily ration was made up from ensilaged maize and alfalfa products, alfalfa and grass hay and cereals completed with vitamins and minerals, in accordance with the NRC (1989) recommendations.

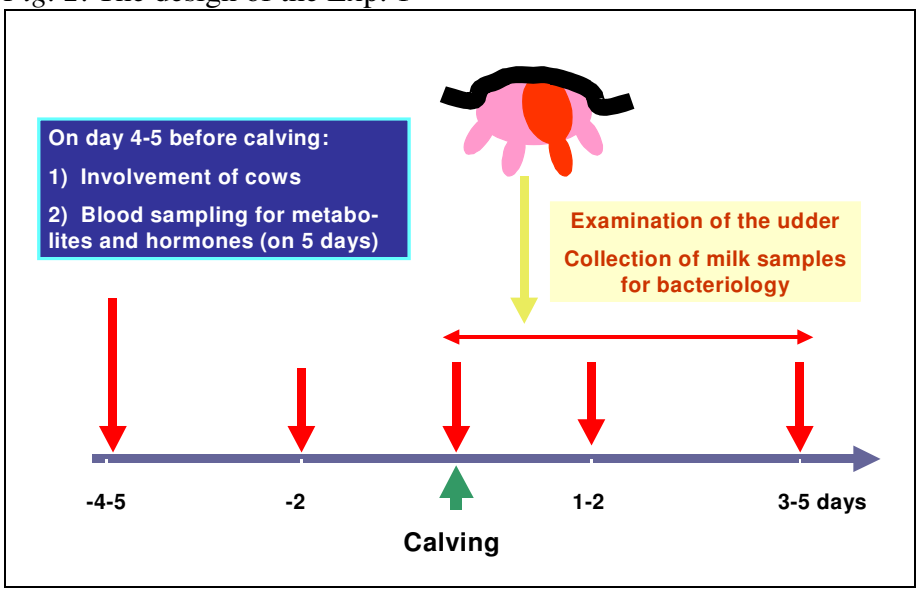
In order to avoid the age-related interference only ≥2 parity cows were involved in the trial. The course of calving was normal in all of them.

4.2.2.2. Experimental animals. Design

Exp. 1 (in Herd A only)

Fifteen cows calving within a 3-week period in September - October 1999 were selected for this trial some days before their expected delivery. No clinical symptoms of any diseases had been observed in these cows during the previous 60-day dry period. Blood samples for endocrine and metabolite assays were taken from them at 8.00 h AM on days 4-5 and 2 prepartum and on days 1-2 and 3-5 postpartum, as well as at the onset of delivery on the day of parturition. The cows calved, and were kept separately in groups of 3-4 individuals in the maternity unit of the farm during the whole sampling procedure. If outbreak of mastitis was observed *simultaneously with calving or in the first 5 days postpartum*, a standard procedure consisting of clinical examination, milk sampling for bacteriology and therapy was carried out. At the end the data of cows affected vs. not affected by various forms of mastitis were compared. (*Fig. 2*)

Fig. 2. The design of the Exp. 1



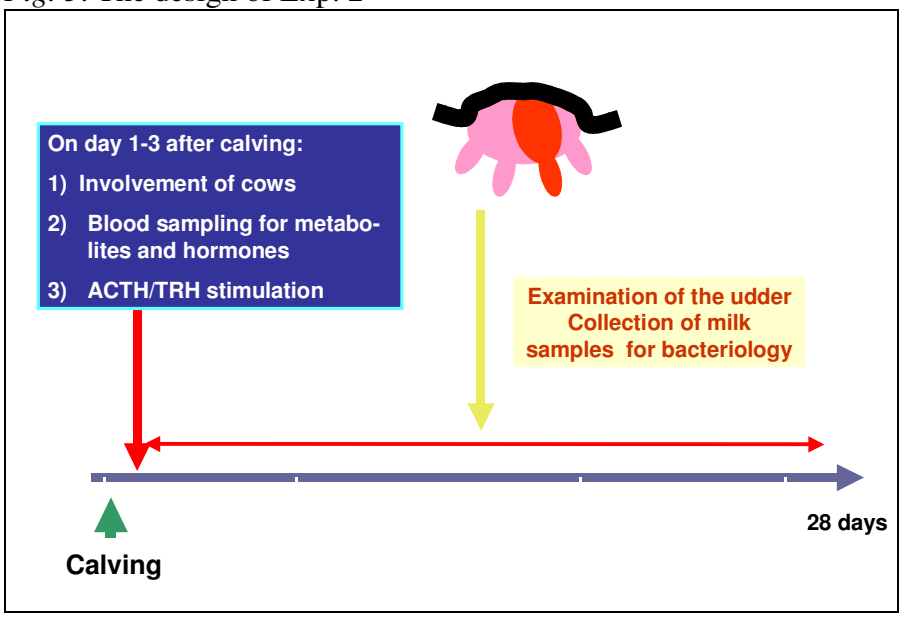
Exp. 2 (in all of the four herds)

All of cows which (1) were free from any clinical symptoms of chronic mastitis (in addition in Herds B and C: did not show positivity in any of their udder quarters with California Mastitis Test in the first 1-3 d postpartum), and (2) calved within the pre-selected periods of the study (Herd A and B: October - November, 1996; Herd C: May - June, 1997; Herd D: July - August, 1998 and 1999) were involved in the trial, unless they needed veterinary intervention at calving, calved twins, and/or showed clinical symptoms of parturient paresis, hepatic injuries or mastitis before taking blood samples on d 1-3 after calving.

During these pre-selected study periods the farms were visited twice a week and all the cows (n = 335) calved 1 to 3 days before and met the above requirements were enrolled in the study. 60 to 90 min. after the morning milking (and before the morning feeding) a blood sample was taken from each of them for determination of certain hormones, metabolites and enzymes known to be related with the energy metabolism and liver function. Also the adrenocorticotrop hormone (ACTH) challenged cortisol release and the thyreotrop-releasing hormone (TRH) induced T_4 and T_3 responses were determined. (The cows in Herds A, B and C were sampled again in further 4 occasions 7 days apart. However, due to the inconsistent tendencies in hormone and metabolite levels determined in these samples the corresponding data were excluded from the final evaluation, and the cows in Herd D were sampled only once, e.g. immediately after their calving.)

If after this day 1-3 sampling procedure mastitis was diagnosed *in the first 28 days* of lactation, the standard clinical examination, milk sampling for bacteriology and treatment were performed. At the final evaluation data of non-mastitic cows were compared to those with mastitis caused by groups of the various pathogens. (*Fig. 3*) The course of uterine involution was checked by rectal palpation and vaginoscopy in all cows on day 6-14 after calving. The malodorous, reddish-brown, watery character of vaginal discharge was considered to be the clinical proof of *acute putrid endometritis* (APE), and the cows showing these pathognostic symptoms were assigned into a separate group.

Fig. 3. The design of Exp. 2



Exp. 3 and *4* (in Herd A and D)

These two studies were conducted in cows calved in April - June, 1997 (Herd A) and in May - July, 1998 and 2000 (Herd D). All of them were milked 3 times a day (between about 8.00 - 9.30 h, 15.30 - 17.00 h and 23.00 - 0.30 h) during at least the first two months of lactation. Animals affected by mastitis in the *early* and *late puerperium*, or during their *peak lactation* (e.g. on day 0-14, 15-28 or 29-60 after calving, respectively) were enrolled in the study.(*Fig. 4*) In *Exp. 3* also the presence of systemic symptoms (*Table 2*) of this disease was involved in the inclusion criteria. As controls, their healthy counterparts (being almost identical in parity, stage of lactation and current milk yield) were selected in the same herd in both of these experiments. Cows recognized to show the first clinical symptoms of mastitis *at the morning milking* (e.g. at about 8.00 - 9.30 h), and their healthy counterparts were separated for the trials. They were placed and kept in the sanitary unit of the farm until their complete clinical recovery and the end of the withdrawal period of the medicine administered (mastitic cows), or until the sampling process was completed (healthy controls). After diagnosing the outbreak of mastitis the standard clinical examination was performed, milk samples were taken for bacteriology and treatment was administered when the affected cows arrived at the sanitary unit (e.g. at about 10.00 - 11.30 h). In *Exp. 3* blood samples were taken for endocrine and metabolite determinations at 14.00 h, and again at further 5 subsequent times 6 h apart (e.g. at 20.00 h, 02.00 h, 8.00 h, 14.00 h and 20.00 h). (*Fig. 5*) All samplings were preceded by complete milking out at least 90 min. earlier. Simultaneously with this blood sampling milk samples were also taken from the non-affected quarters for assaying the progesterone (P_4) content (in cows after the colostral period only). In *Exp. 4* the ACTH challenged cortisol release and the TRH induced T_4 and T_3 responses were determined (administering the ACTH / TRH at 14.00 h). Only the challenging and sampling processes were performed, however, in the healthy counterparts.

Fig. 4. The design of Exp. 3 and 4

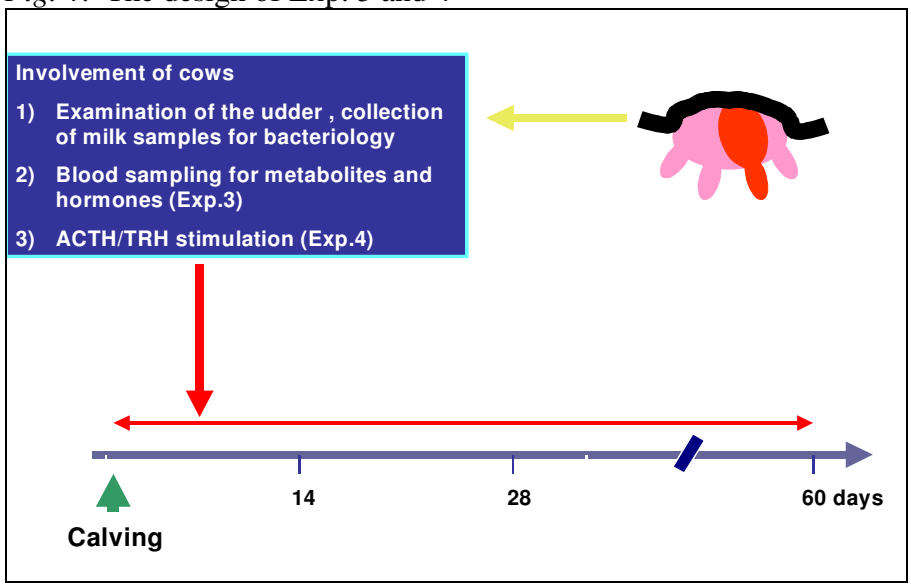
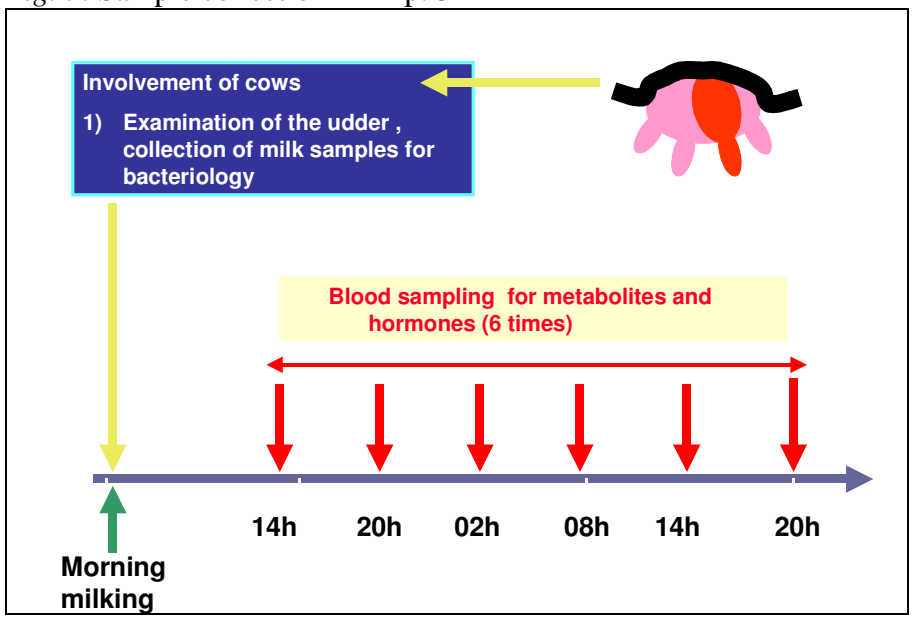


Fig. 5. Sample collection in Exp. 3



4.2.2.3. Clinical examination. Sampling for bacteriology. Isolation and identification of pathogens

As symptoms of mastitis (macroscopic appearance of milk and/or local symptoms on the affected quarter, with or without systemic signs) were observed in cows involved in the trial, the affected individual was separated immediately for clinical examination, sampling for bacteriology and receiving the standard treatment procedure. The date of outbreak and the severity of symptoms were recorded and scored on a scale from 1 (no changes) to 3 (severe reaction) as described by Pyörälä and Syvajarvi (1987) (*Table 1*).

Table 1: Recording and scoring the clinical symptoms of mastitis (after Pyörälä and Syvajarvi, 1987)

	Score 1	Score 2	Score 3
Systemic signs	None	Rectal temperature: ≤ 40.5 C^o and/or slight anorexia and depression	Rectal temperature: >40.5 C^o and/or severe anorexia and depression, or recumbency
Local signs	None	Moderate swelling + tenderness of the affected quarter(s)	Severe swelling, firmness, the quarter very sore to touch
Milk appearance	Normal	Slightly watery, discolored, and/or clots and flakes	Consistency serum-like, pus-like, and/or bloody

Notes:		**Systemic signs**	**Local signs**	**Milk appearance**
Clinical mastitis,	**mild**:	Score 1	Score 1 or 2	Score 2
	moderate:	Score 1 or 2	Score 2 or 3	Score 2 or 3
	severe:	Score 2 or 3	Score 2 or 3	Score 2 or 3

Thereafter aseptic milk samples were taken from their affected quarter(s). The samples were stored frozen at -20 C^o and transported to the lab once (Exp. 1, 3 and 4) or twice (Exp. 2) a week. The mastitis pathogens were isolated after Honkanen-Buzalski and Seuna (1995). From each sample, 0.01 ml was streaked onto the surface of one-fourth plate of 90 mm Columbia agar (Merck, KgaA, Darmstadt, Germany) containing 5% sheep blood and 0.01% esculin. All the plates were incubated at 37 ^oC, and were evaluated after 14 to 16 h and again following an additional 24 h. The colonies were tentatively identified according to their morphology, pigment production, Gram-staining, catalase test and the type of hemolysis produced. The pure cultures were identified based on the recommendations of Honkanen-Buzalski and Seuna (1995) and Quinn et al.(1994).

4.2.2.4. Standard treatment procedure

As usual under farm conditions, schematic intramammary administration of commercially available antimicrobials (e.g. 75 mg Ampicillin Na + 200 mg Cloxacillin Na: Ampiclox L.A.™, Pfizer AH; 235 mg Cephacetril Na: Vetimast™, Novartis AH; 250 mg Cephoperasone: Pathozone™, Pfizer AH) were used for treatment. According to the medical instructions the preparations were administered twice (Ampiclox) or once (Vetimast) a day for 1-4 days, or only once (Pathozone) just after the mastitis outbreak. The systemic administration of glucocorticoids and non-steroidal antiinflammatory agents were not allowed in this study, because we wanted to avoid their interference with the endocrine consequences of mastitis.

4.2.2.5. Blood sampling. Endocrine and metabolic assay procedures

To assay the *basal* concentrations of hormones and metabolites blood (hereafter: t_0) samples were collected in accordance with the design of *Exp. 1-4* (for details: see there) from the jugular vein into heparinized (in *Exp. 1-4*) and NaF containing tubes (in *Exp. 2* only). The samples were centrifuged within 30 min.. The NaF plasma was kept on +4 C^o and assayed for glucose within 24 h (in *Exp. 2* only). The heparinized plasma was stored frozen at -20 C^o until assaying for other metabolites and hormones.

The levels of acetoacetic acid (AcAc), βOH-butyrate (BHB), non-esterified fatty acid (NEFA), trigliceride (TG), total cholesterol (TCh) and urea, as well as of progesterone (P_4), cortisol, thyroid hormones [T_4, T_3 and the inactivated thyroid hormone formation (3,3',5'-triiodothyronine or reverse-triiodothyronine, rT_3)], insulin, IGF-I and IGF-II were analyzed. The parameters determined in *Exp. 1* to *4*, and the analytical procedures are summarized in *Table 2* and 3.

Table 2: The endocrine assay procedures

Hormone	Technique	Trial	Sensitivity [a]	Intraassay CV %	Interassay CV %	Kit / Literature
Hormones from defatted milk samples [b]						
Progesterone (P_4)	Microplate ELISA [c]	Exp. 3	0.18 nmol/l	5.1 - 11.6	≤12.3	Locally developed assay (Budapest) (Nagy et al, 1998)
Hormones from blood (heparinized plasma) samples [b]						
Progesterone (P_4)	Direct ^{3}H-RIA [d]	Exp. 1 and 3	0.58 nmol/l	5.3 - 11.6	6.8 - 12.3	Locally developed assay (Budapest) (Nagy et al, 1998)
Cortisol	Direct ^{3}H-RIA [e]	Exp. 1, 2, 3 and 4	0.27 nmol/l	3.2 - 8.7	≤ 10.3	Locally developed assay (Budapest) (Csernus, 1982)
Thyroxin (T_4)	^{125}I-RIA	Exp. 1	2.00 nmol/l	3.6 - 4.2	4.3 - 9.5	^{125}I-T_4 RIA kit (INEP, Zemun, Yugoslavia)
		Exp. 2, 3 and 4	1.46 nmol/l	6.6 - 8.5	≤ 7.7	^{125}I-T_4 RIA MIS kit [f] (Institute of Isotopes Co. Ltd, Budapest, Hungary)
3,3',5-triiodothyronine (T_3)	^{125}I-RIA	Exp. 1	0.25 nmol/l	5.2 – 7.2	4.1 – 9.3	^{125}I-T_3 RIA kit (INEP, Zemun, Yugoslavia)
		Exp. 2, 3 and 4	0.18 nmol/l	6.2 - 8.8	≤ 6.7	^{125}I-T_3 RIA MIS kit [g] (Institute of Isotopes Co. Ltd, Budapest, Hungary)
3,3',5'-triiodothyronine (rT_3) [h]	^{125}I-RIA	Exp. 2 and 4	25.75 pmol/l	5.4 - 7.8 %	≤ 10.3	Locally developed assay (Budapest) [i]
Insulin	^{125}I-RIA	Exp. 1	0.60 µIU/l	2.5 – 5.2	7.7 – 10.7	^{125}I-Insulin RIA kit (INEP, Zemun, Yugoslavia)
		Exp. 2 (samples taken in *Herds A-B*)	1.04 µIU/ml	7.7 - 10.2	≤ 12.3	^{125}I-Insulin RIA PEG kit [j] (Institute of Isotopes Co. Ltd, Budapest, Hungary)
		Exp. 2 (samples taken in *Herds C-D*), 3 and 4	1.08 µIU/ml	5.5 - 8.4 %	≤ 8.8	^{125}I-Insulin RIA CT kit [j] (CIS Bio International Ltd, Gif-Sur-Yvette, France)
Insulin-like growth factor-I (IGF-I)	Extraction and heterologous ^{125}I-RIA [k]	Exp. 2 (samples taken in *Herds A-B*) and 3	0.25 nmol/l	3.6 - 6.5 %.	≤ 12.5	Locally developed assay (Utrecht) (Nap et al, 1993) [k, l]
		Exp. 1, 2 (samples taken in *Herds C-D*)	0.20 nmol/l	3.1 – 6.2	≤ 12.0	Locally developed assay (Zemun) (Nikolić et al. 1996, 2001a) [k, l]
Insulin-like growth factor-II (IGF-II)	Extraction and heterologous ^{125}I- RIA [k]	Exp. 1	0.31 nmol/l [m]	3.0 – 6.0	5.0 – 13.0	Locally developed assay (Zemun) (Nikolić et al. 1998, 2001a) [k, m]

Notes for *Table 2*:

[a] Lower limit of sensitivity

[b] In all of the assay systems the binding pattern of serially diluted cow samples was parallel to that of the standard curves, and the recovery of added hormones from bovine milk / plasma varied between 94 and 106 %.

[c] Modified and validated for assaying P_4 in bovine skimmilk (Huszenicza et al., 1998; Taponen et al., in press.).

[d] Validated for assaying P_4 in bovine plasma.

[e] Validated for assaying cortisol in bovine plasma (Kulcsar et al, 1996).

[f] The kit developed for human use was slightly modified and validated for assaying T_4 in bovine plasma.

[g] The kit developed for human use was validated for assaying T_3 in bovine plasma.

[h] Synonym: reverse-triiodo-thyronine (rT_3)

[i] A locally developed ^{125}I-RIA system based on the use of a specific antibody, as well as labeled and unlabeled rT_3 provided by the of *Institute of Isotopes Co. Ltd* (*Budapest, Hungary*) (the cross-reaction of antibody with T_4 and T_3 was <1 %). The antibody bound and free phases were separated with dextran charcoal technique.

[j] The kit developed for human use was validated for assaying insulin in bovine plasma.

[k] Total concentrations of IGF-I and IGF-II were determined in neutralized acid-ethanol extracts of plasma using classical ^{125}I-RIA systems, as slightly modified and validated for bovine samples (in Utrecht: Dieleman, unpublished; in Zemun: Nikolić et al. 1996, 2001a).

[l] Since the amino acid composition of bovine (b) and human (h) IGF-I is the same, h-IGF-I was used as the working standard in the presence of h-IGF-II (4 ng/tube).

[m] The IGF-II RIA was calibrated against reference h-IGF-II (WHO 96/538) but partially purified b-IGF-II was used as the working standard (Nikolić et al. 1998, 2001a). So the results are expressed in human equivalent (HE) nmol/l.

Table 3: The assay procedures used for determination of enzymes and metabolites

Parameter		Technique	Kit / Literature
Aspartate aminotransferase (AST)	Exp. 2	IFCC, UV method*	Reanal Ltd, Budapest, Hungary (Kit 7249)
Glucose	Exp. 2	Enzymatic (GOD-POD reaction*	Diagnosticum Ltd, Budapest, Hungary (Kit 40841)
Acetoacetate (AcAc)	Exp. 2	Salicylaldehyde reaction	Walker, 1954
βOH-butyrate (BHB)	Exp. 1, 2 and 3	βOH-butyrate dehydrogenase reaction*	Sigma Diagnostics Co., (Kit 310-A)
Non-esterified fatty acid (NEFA)	Exp. 1, 2 and 3	Extraction of their colored soaps	Noma et al., 1973
Total cholesterol (TCh)	Exp. 2	Enzymatic (CHOD-PAP) reaction *	Diagnosticum Ltd, Budapest, Hungary (Kit 40121)
Trigliceride (TG)	Exp. 2	Enzymatic method*	Diagnosticum Ltd, Budapest, Hungary (Kit 40721)
Urea	Exp. 2	Enzymatic (urease) reaction	Reanal Ltd, Budapest, Hungary (Kit 16571)

Notes: *Measured on an automathized clinico-chemical analyzer (Eppendorf ACP 5040)

4.2.2.6. Challenge tests of adrenocortical and thyroid function. (in Exp. 2 and 4)

The adrenocortical and thyroid response to standard low doses of ACTH and TRH challenges were determined after Kolk (1991) and Tveit et al. (1990), respectively. Immediately after taking the t_0 samples 60 µg $_{1\text{-}24}$ACTH (*Cortrosin inj™*, Organon, Oss, The Netherlands) and 400 µg TRH (*pGLU-HIS-PRO amide acetate salt*, Sigma, St. Louis, USA) solved in saline were administered into the jugular vein simultaneously (but from two different syringes), and another blood samples were collected again 60, 240 and 360 minutes later (t_{60}, t_{240} and t_{360} samples, respectively) for cortisol (in t_{60} sample) and T_4 and T_3 determinations (in t_{240} and t_{360} samples).

4.2.2.7. Data evaluation

In *Exp. 2* the 1.00 mmol/l of BHB level was estimated as a border between *hyperketonemic* (≥1.00 mmol/l) and *normoketonemic* (<1.00 mmol/l) conditions (Bruss, 1997). When the physiological degree of ACTH induced cortisol response was calculated, the data (x ± SD) on cortisol determined in t_{60} samples of symptomless, normoketonemic (n = 199) cows were used: the value of standard deviation (SD; 49.59 nmol/l) was subtracted from the mean level (x; 89.16 nmol/l). After Kolk (1991) the case of *temporary hypocorticism* was supposed, when the current t_{60} cortisol value of a cow was below this calculated threshold of about 40 mmol/l.

The results were subjected to chi square test (distributions), Student's t test (pair-wise comparison of group means) or one-way analysis of variance (ANOVA) (comparison of ≥ 3 groups). The statistical significance of differences between means of ≥ 3 groups was estimated by calculating the least significant difference (LSD). In *Exp. 1* also split-plot factorial analysis of variance was done when appropriate (two missing values were calculated and the degrees of freedom adjusted accordingly) (Kleinbaum and Kupper, 1978; Juvancz and Paksy, 1981). Some correlation and multiregression analyses were also performed. For estimation of prognostic value attributable to circulating hormone and metabolite levels being above/below a given threshold the Odds ratio was calculated in *Exp.2*. Considering pathogen *types 1* (contagious pathogens: *S. aureus*), *2* [*Gram-positive* (GP) *environmental pathogens*], and 3 [*Gram-negative* (GN) *environmental pathogens + mastitis caused by not detectable pathogens* (NDP)] separately, stepwise logistic regression was used to analyze the relation between potential prognostic factors (hormone and metabolite levels) and mastitis outbreak. Analyses were made by the statistical package SPSS for Windows 8.0.

4.2.3. RESULTS

4.2.3.1. Effect of mastitis on peri-parturient endocrine changes (Exp. 1)

Clinical findings

During the peri-parturient sampling period no inflammatory processes were detected in five animals (Cows **323, 3092, 3122, 6942** and **7044**), which remained healthy throughout the subsequent lactation.

Mastitis associated with GP contagious pathogens (*S. aureus*) appeared in two cows on day 1 and day 5 postpartum, respectively (*Table 4*). In the first case (Cow **5599**), the calf was stillborn and all four udder quarters became severely affected. Only two quarters of Cow **5896** were affected, the calf was healthy but fetal membranes were retained for 2 days after calving. Despite treatment the mastitis became chronic and relapsing, so the cows were eventually culled.

On days 1 to 3 after calving mastitis was diagnosed in a further eight cows (*Table 4*). In six of them GN bacteria (mainly *E. coli*) were identified as single pathogens, or in combination with coagulase-negative *Staphylococci* (CNS). The fetal membranes were retained for 2 days in two of these cows (Nos **979** and **5198**). In the two other cases (Cows **643** and **5929**) *no detectable pathogen* (NDP) could be isolated from the mastitis affected quarters, although CNS were identified in one inflamed quarter of Cow **5929**. Despite the severe general and local symptoms observed in Cows **5198** and **7097**, all the animals showed complete clinical recovery within 2-5 days.

When endocrine and metabolic changes were evaluated, the mastitic cows (*Table 4*) were placed in subgroups of cases caused by *GN environmental pathogens* (n = 6; five of them resulted from intramammary *E. coli* infection), *NDP mastitis* (n = 2) or *GP contagious pathogens* (*S. aureus*; n = 2). Mastitis related endotoxin loading was considered to be confirmed, supposed or excluded in these subgroups, respectively. Unfortunately the limited number of cows available in the *Exp. 1* allowed the realistic statistical comparison of healthy cows and of those with *E. coli* mastitis only.

Endocrine and metabolic characteristics of healthy cows and of those with GN mastitis

The time course of changes in mean hormone and metabolite concentrations for the two groups of five cows is given in *Table 5*. There were highly significant time-associated changes for P_4, T_3, T_4, IGF-I, IGF-II, cortisol, insulin, BHB and NEFA concentrations. One control cow (No **3092**) showed obviously elevated lipid mobilization (NEFA: 1.09 mmol/l) and mild hyperketonaemia (BHB: 1.35 mmol/l) on day 2 postpartum but all other values for BHB were below the physiological limit (<1 mmol/l). A statistically significant difference between the groups was detected only for T_4 and BHB, because individual characteristics of the cows and the small number of individuals prevented the apparently large difference in mean IGF-I from showing statistical significance ($P = 0.13$).

The presence of significant time/group interactions for T_3 and IGF-II indicated that the changes with time were not the same for both groups (*Table 5*). Namely, while the mean concentrations of T_3 and IGF-II were similar prepartum, lower concentrations were recorded postpartum in the cows exhibiting mastitis. Although the significant decrease in mean plasma IGF-II associated with parturition was of similar magnitude (~7 nmol HE/l) in both groups, low concentrations remained on day 2 postpartum in the cows with mastitis.

Table 4: The clinical findings in cows with mastitis in *Exp. 1*

Cow No	General symptom score	Left forequarter				Right forequarter				Left rearquarter				Right rearquarter			
		Day of outbreak	Pathogen	Score of local symp.	Score of Milk appear.	Day of outbreak	Pathogen	Score of local symp.	Score of milk appear.	Day of outbreak	Pathogen	Score of local symp.	Score of milk appear.	Day of outbreak	Pathogen	Score of local symp.	Score of milk appear.
Mastitis with *Gram-negative environmental pathogens* (as a single pathogen or in combination with others; endotoxin loading confirmed)*																	
979	1					2	*Klebs.*[1]	2	3								
5198	3	1	*E.coli*	2	3	1	*E.coli*	1	2	1	*E.coli*[2]	2	3	3	CNS	1	2
6011	1					1	*E. coli*	2	2	1	CNS	1	2				
6204	2					1	*E. coli*	2	3	1	CNS**	1	1	1	CNS	1	2
7063	2					2	*E. coli*	2	3	2	*E. coli*	1	2	3	*E. coli*[3]	1	2
7097	3	3	CNS	1	2	1	*E. coli*	3	3	1	*E. coli*	2	3				
Mastitis with *no detectable pathogens* (NDP) in at least one quarter (endotoxin loading supposed)*																	
643	2									1	NDP.	2	3	2	NDP.	2	3
5929	2					2	NDP.	2	3	2	CNS	1	2				
Mastitis with *Gram-positive contagious pathogens* (endotoxin loading excluded)***																	
5599	2	1	S. aur.	2	2	1	*S. aur*	1	2	1	*S. aur*	3	3	1	*S. aur*[1]	2	2
5896	2	5	S. aur	2	2					5	*S. aur*	3	3				

Notes: *Klebs.: Klebsiella oxytoca; E. coli: Escherichia coli*; CNS: coagulase-negative *Staphylococci; S. aur.: Staphylococcus aureus*; NDP: no detectable pathogens

[1,2,3]Besides the primary pathogen some colonies of [1]CNS (Cows 979 and 5599), [2]*Klebsiella* (Cow 5198), or [3]*Serratia* (Cow 7063) strains were also isolated

*Complete clinical recovery was observed in all of the affected quarters within 3-4 days.

**Despite the positive bacteriological finding, no clinical symptoms were seen in the quarter.

***Both cases became chronic, relapsing again on days 21 (Cow 5599) and 31 (Cow 5896). The cows were culled after the clinical relapse.

Table 5: Mean concentrations of hormones in plasma of cows *with no clinical signs of mastitis during the early puerperium* (Contr.; n=5), and of those with episodes of post-parturient *E. coli* mastitis (Mast.; n=5) in *Exp. 1*.

		Mean time from calving (days)					F-	F-	F-
		-5.0	-2.2	0	2.0	4.8	group	time	int.
P4 (nmol/l)	Contr.	5.42^{a}	4.71^{a}	0.87^{b}	0.52^{b}	0.55^{b}	*1.03*	*59.6****	*1.23*
	Mast. ♦	4.01^{a}	4.67^{a}	0.82^{b}	0.73^{b}	0.38^{b}			
T3 (nmol/l)	Contr.	1.48^{ab}	1.72^{ab}	1.44^{ab}	1.26^{bc}	1.31^{abc}	*0.44*	*9.14****	*2.75* *
	Mast. ♦	1.79^{a}	1.51^{ab}	1.41^{ab}	0.91^{c}	0.89^{c}			
T4 (nmol/l)	Contr.	42.2^{ab}	44.2^{a}	29.5^{de}	33.3^{bcde}	42.0^{abc}	*6.27**	*8.85****	*0.94*
	Mast. ♦	37.5^{abcd}	31.3^{cde}	24.7^{e}	23.5^{e}	30.7^{de}			
IGF-I (nmol/l)	Contr.	14.5^{a}	10.8^{ab}	8.5^{ab}	6.6^{b}	6.2^{b}	*2.82*	*4.42***	*0.49*
	Mast. ♦	7.9^{ab}	8.7^{ab}	5.8^{b}	3.8^{b}	3.8^{b}			
IGF-II (HE nmol/l)	Contr.	17.8^{bcd}	15.3^{cde}	8.1^{e}	20.9^{abc}	26.3^{a}	*0.01*	*11.83****	*3.18* *
	Mast. ♦	18.7^{abcd}	19.6^{abcd}	12.5^{de}	12.5^{de}	23.6^{ab}			
Cortisol (nmol/l)	Contr.	3.1^{b}	2.8^{b}	11.4^{a}	3.4^{b}	3.0^{b}	*0.49*	*15.56****	*0.51*
	Mast. ♦	3.9^{b}	3.5^{b}	12.8^{a}	5.7^{b}	1.5^{b}			
Insulin (mIU/l)	Contr.	6.4^{ab}	6.3^{ab}	2.3^{c}	3.9^{abc}	4.8^{abc}	*0.54*	*5.13****	*1.99*
	Mast. ♦	7.0^{a}	4.0^{abc}	2.9^{abc}	6.5^{ab}	6.8^{a}			
BHB (mmol/l)	Contr.	0.43^{bc}	-	0.46^{abc}	0.80^{a}	0.75^{ab}	*5.27**	*4.49**	*1.83*
	Mast. ♦	0.36^{c}	-	0.39^{c}	0.46^{abc}	0.43^{bc}			
NEFA (mmol/l)	Contr.	0.27^{b}	-	0.49^{ab}	0.69^{a}	0.54^{ab}	*0.05*	*5.19***	*0.22*
	Mast. ♦	0.40^{ab}	-	0.59^{ab}	0.51^{ab}	0.57^{ab}			

Notes: ♦Their mastitis related endotoxin loading was considered to be confirmed

^{abcde}Means for the same substance not sharing superscripts are significantly different (P<0.01)

*P<0.05

**P<0.01

***P<0.001

Table 6: Mean concentrations of hormons in plasma of cows with episodes of postparturient mastitis without identified pathogen (NDP mastitis) and of those with *S. aureus* mastitis in *Exp. 1*

	Mean time from calving (days)				
	-5.0	-2.2	0	2.0	4.8
Mastitis with no detectable pathogens (NDP mastitis) (n=2)					
P4 (nmol/l)	4.67	5.37	1.05	0.37	0.56
T3 (nmol/l)	1.18	1.06	0.75	0.71	0.88
T4 (nmol/l)	32.2	27.2	23.0	23.6	34.9
IGF-I (nmol/l)	5.6	5.2	5.9	6.7	4.1
IGF-II (HE nmol/l)	16.8	14.1	6.6	18.9	21.1
Cortisol (nmol/l)	4.2	4.4	12.4	3.5	1.3
Insulin (mIU/l)	4.2	5.1	2.4	3.7	6.6
BHB (mmol/l)	0.57	-	0.70	0.73	0.79
NEFA (mmol/l)	0.48	-	0.95	0.75	0.65
S. aureus mastitis (n=2)					
P4 (nmol/l)	4.02	3.31	0.73	0.59	0.47
T3 (nmol/l)	1.87	1.28	1.39	1.21	1.38
T4 (nmol/l)	45.5	34.3	27.2	36.0	29.6
IGF-I (nmol/l)	12.2	13.5	10.0	11.6	8.4
IGF-II (HE nmol/l)	20.1	21.5	16.7	24.4	29.1
Cortisol (nmol/l)	1.7	2.0	14.4	1.7	0.4
Insulin (mIU/l)	6.1	6.7	3.2	7.2	12.1
BHB (mmol/l)	0.43	-	0.80	0.72	0.52
NEFA (mmol/l)	0.38	-	0.59	0.63	0.35

Endocrine and metabolic characteristics of cows with NDP mastitis or S. aureus associated mastitis

The time course of changes in hormone and metabolite concentrations for these four cows is given in *Table 6*. While the small number of cases prevented the realistic statistical evaluation, it is apparent that the trends for the two cows with supposed Gram-negative mastitis were similar to those where recognized pathogens had been isolated (*Table 5*). Namely, thyroid hormone and IGF-I concentrations were generally low. In contrast, the two cows with mastitis caused by Gram-positive bacteria had peripheral hormone concentrations similar to those with no symptoms of mastitis.

Hormonal interrelationships

Multiregression analysis of the results for the whole period examined, showed that T_4 concentrations overall were independently associated with T_3 ($t = 8.93$; $P < 0.001$) and IGF-I concentrations ($t = 2.28$; $P = 0.025$) positively, together with cortisol negatively ($t = -4.97$; $P < 0.001$; $R^2 = 0.648$), which relationship accounted for two-thirds of the variance in T_4 concentrations. Analysis of the data obtained for the two intervals prepartum gave a similar independent relationship ($R^2 = 0.709$) for T4 with T3 ($t = 5.56$; $P < 0.001$), IGF-I ($t = 3.40$; $P = 0.002$) and cortisol ($t = -2.02$; $P = 0.053$). However, T_4 concentrations postpartum were associated with T_3 ($t = 4.20$; $P < 0.001$) and IGF-II ($t = 1.94$; $P < 0.062$, $R^2 = 0.565$). IGF-I and IGF-II concentrations overall were weakly correlated ($r = 0.289$; $P = 0.012$) but more significant positive correlations emerged when the data obtained prepartum ($r = 0.466$; $P = 0.009$) and postpartum ($r = 0.567$; $P = 0.001$) were analyzed separately.

4.2.3.2. Metabolic predisposition for and clinical characteristics of mastitis observed in early weeks of lactation (Exp. 2)

In the first 4 weeks of their lactation mastitis was observed to occur in 146 of the 335 cows involved in *Exp. 2* (identified pathogens: *Table 7*). Within 1-5 days after the outbreak of mastitis 11 of these 146 cows *died* or were *emergency slaughtered* (thereafter referred to as *lost* cases). Some others were lost only later, after a 10 to 25 day-long course of this disease. In all the lost cows the first pathognostic symptoms of mastitis were recorded in the first 10 days after calving. A*cute putrid endometritis* (APE) was diagnosed in 49 of the 146 mastitic cows on days 6-12. In the overwhelming majority of these 49 cows environmental pathogens were identified (n=32), or no pathogens were detected (NDP mastitis; n=12). Contagious pathogens (represented by *S. aureus*) were found only in 5 cases (*Table 7*). In the group of non-mastitic 187 cows 12 animals were lost (9, 2 and 1 of them due to ketosis plus fatty liver, severe weight loss plus lameness, and APE, respectively).

The severity of clinical symptoms was the same in cows with GN and NDP mastitis, and they showed usually more severe clinical symptoms with higher rectal temperature than the animals with GP mastitis. So the corresponding clinical data of cows with GN and NDP mastitis were pooled and compared to those with GP intramammary infection (IMI) in *Table 8*. In severity of symptoms, however, no significant differences (P>0.1) were found between the cases occurring in the first vs. second 2-week periods.

Table 7: Mastitis pathogens isolated and identified in *Exp. 2*.

	Mastitis on day ≤ 28				
	All cases of mastitis on day ≤ 28	Mastitis as a single disease			Mastitis (on day 1-28) + APE (on day 6-12)
		On day 1-14		On day 15-28♦♦	
		All cases	Of them: lost♦		
S. aureus	30	19	2	6	5
Coagulase-negative Staphylococci (CNS)	8	7	--	1	--
Strep. dysgalactiae	9	4	--	1	4
Strep. uberis	25	9	1	6	10
Other Str.	5	1	--	--	4
E. coli	38	18+	4	8	12♦♦♦
Klebsiella	2	--	--	--	2
No detected pathogen (NDP)	29	12	4	5	12++
All	146	70	11	27	49

Notes: APE: acute putrid endometritis (diagnosed on day 6-12)

♦Lost: number of cows *died* + *emergency slaughtered* due to mastitis within 1-5 days after the outbreak.

♦♦None of the cows was lost due to mastitis within 1-5 days after the outbreak.

♦♦♦2 of them were lost due to mastitis within 1-5 days after the outbreak, and 3 others after a longer (14-21 day-long) course.

+2 other cows were lost after a longer (15-23 day-long) course.

++3 of these cows were lost after a longer (10-25 day-long) course.

Table 8: Severity of clinical symptoms in mastitis caused by various pathogens in the first and second 2-week periods after calving (*Exp. 2*).

		Rectal temperature, °C	Score of systemic signs	local signs	milk appearance	altogether
Mastitis on d 1-14						
GP (n=40)	x	39.7	1.4	1.8	2.1	5.2
	± SD	0.6	0.7	0.7	0.3	1.4
GN+NDP (n=30)	x	40.7	2.7	2.4	2.4	7.4
	± SD	0.8	0.5	0.6	0.5	1.3
P<		*0,001*	*0,001*	*0,001*	*0,01*	*0,001*
Mastitis on d 15-28						
GP (n=14)	x	39.5	1.6	1.8	2.1	5.5
	± SD	0.7	0.6	0.7	0.4	1.5
GN+NDP (n=13)	x	40.4	2.5	2.5	2.4	7.3
	± SD	0.5	0.7	0.5	0.5	1.3
P<		*0,001*	*0,01*	*0,01*	*ns*	*0,01*

Notes: GN: Gram-negative; GP: Gram-positive; NDP mastitis: mastitis with no detected pathogens

Table 9: The plasma metabolite and hormone levels of non-mastitic cow and of those with mastitis on d 1-14, and d 15-28, as a single disease, or in combination with acute putrid endometritis (APE) (*Exp. 2*).

	AcAc (mmol/l)	BHB (mmol/l)	NEFA (mmol/l)	IGF-1 (nmol/l)	Insulin (μIU/ml)	T_4 (nmol/l)	T_3 (nmol/l)	rT_3 (pmol/l)
Non-mastitic	0.132^{a}	0.89^{abc}	0.358^{abc}	4.72^{abc}	3.87	28.84^{ab}	1.18	217^{ab}
(n=187)	0.086	0.56	0.217	1.77	2.39	9.35	0.29	58
Mastitis, d 1-14	0.150^{b}	1.12^{a}	0.447^{a}	$3,99^{a}$	3.43	25.54^{a}	1.10	233
(n=70)	0.114	0.58	0.221	1,57	2.46	6.02	0.18	43
Mastitis, d 15-29	0.273^{abc}	1.18^{b}	0.454^{b}	$4,02^{b}$	3.24	26.68	1.10	236^{a}
(n=27)	0.394	0.50	0.261	1,59	3.03	5.08	0.19	36
Mastitis + APE	0.157^{c}	1.15^{c}	0.436^{c}	$4,12^{c}$	3.65	25.69^{b}	1.11	237^{b}
(n=49)	0.124	0.68	0.219	1,50	2.24	6.48	0.22	48
F=	*7.33*	*5.22*	*4.21*	*4.56*	*0.91*	*4.01*	*2.52*	*3.27*
$LSD_{(P<0.05)}$=	*0.054*	*0.21*	*0.082*	*0.60*	*ns*	*2.99*	*ns*	*19*

Note: ^{abc}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 10: The plasma metabolite and hormone levels in cows affected by *contagious* pathogens (*S. aureus*) vs. *environmental* pathogens plus *NDP* on d 1-14 as a single disease, or in combination with acute putrid endometritis (APE) (*Exp. 2*).

	AcAc (mmol/l)	BHB (mmol/l)	IGF-1 (nmol/l)	Insulin (μIU/ml)	T_4 (nmol/l)	T_3 (nmol/l)	rT_3 (pmol/l)
Mastitis caused by *S. aureus*	0.094	0.80	4,52	5.35	27.17	1,22	0.22
(n=24)	0.076	0.34	1,58	2.57	7.25	0,22	0.06
Mastitis caused by GP + GN environmental pathogens + NDP (n=95)	0.168	1.21	3,80	3.06	25.19	1,06	0.24
	0.122	0.65	1,55	2.08	5.86	0,16	0.04
P<	*0.001*	*0.001*	*(0.1)*	*0.001*	*(0.1)*	*0.01*	*(0.08)*

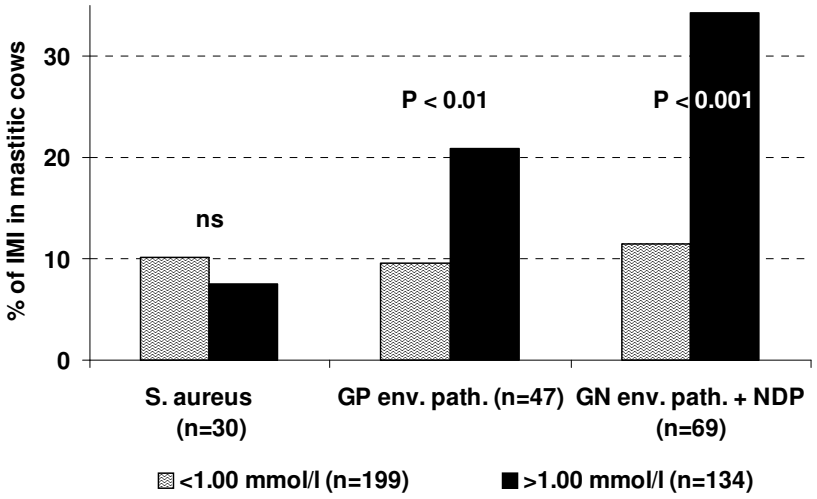
Table 11: The predictive value of *day 1-3 hyperketonaemia* (BHB level: ≥1.00 mmol/l) for occurrence of clinical mastitis caused by various pathogens in the first 4 weeks of lactation (*Exp. 2*).

Isolated mastitis pathogen	Odds ratio	95 % confidence interval Lower	Upper
Mastitis outbreak: *on d 1-28 after calving*			
S. aureus	1.333	0.585	3.041
GP environmental pathogens	3.600	1.857	6.977
GN environmental pathogens + NDP	5.333	2.941	9.670

Notes: GN: gram-negative; GP: gram-positive; NDP mastitis: mastitis with no detected pathogens

On day 1-3 after calving the cows affected by mastitis later in the subsequent 4 weeks (as a single disease, or in combination with APE) were characterized by significantly elevated AcAc, BHB, NEFA and rT_3 levels, and lower IGF-1, T_4 and T_3 concentrations then their non-mastitic herd mates (*Table 9*). No similar mastitis related significant differences were found, however, in glucose, TCh, urea and insulin concentrations, as well as in TRH induced T_4 and T_3 responses (details are not given). These metabolic and endocrine differences derived mainly from data of cows infected with GP (CNS, *Strep. dysgalactiae*, *Strep. uberis*, and other *Strep*.) and GN (*E. coli* and *Klebsiella* strains) environmental pathogens later,

Fig. 6: The βOH-butyrate related distribution of mastitic cases caused by *S. aureus*, GP and GN *environmental pathogens* plus those with *no detected pathogens* (NDP) in the first 4 weeks of lactation (*Exp. 2*). (Note: 5, 7 and 12, as well as 0, 11 and 14 of these cases were affected also by *acute putrid endometritis* in the normoketonemic and hyperketonemic cows, respectively.)



plus from the mastitic cases with *no detected pathogens* (NDP), rather than from those with *S. aureus* mastitis (*Table 10*). In the first 4 weeks of lactation the occurrence of *S. aureus* mastitis was almost the same in hyperketonemic (BHB level: ≥1.00 mmol/l) as in normoketonemic (BHB level <1.00 mmol/l) cows. However, the prevalence of mastitis caused by *GP* and *GN environmental pathogens* plus of those with *NDP mastitis* was significantly higher among the hyperketonemic individuals (*Fig. 6*). In mastitis caused by environmental pathogens clear hyperketonaemia related predisposition was verified also by calculating the odds ratio (*Table 11*). The hyperketonaemia based prediction was more pronounced in GN plus NDP than GP mastitis. However, no significant forecasting value was attributable to BHB elevation in *S. aureus* mastitis (*Table 11*). However, despite the significant differences in day 1-3 mean concentrations (*Tables 9* and *10*), due to their low odds ratio no obvious predictive value can be attributed to any other hormones or metabolites, and neither hyperketonaemia, nor the other determined parameters related directly to severity and course in any forms of mastitis (no details are given).

4.2.3.3. Involvement of temporary hypocorticism in pathogenesis of mastitis (Exp. 2)

When the day 1-3 characteristics of adrenocortical function was evaluated, no mastitis related differences were found in the *basal cortisol* levels. Based on their above normal cortisol response to a standard low dose ACTH challenge *temporary hypocorticism* was diagnosed in 76 of the 335 cows (22.7 %). In the first two weeks 7 and 10 of them (altogether 22.4 %) were affected by GP and GN+NDP mastitis, as a single disease. The cows with GN or NDP mastitis showed more severe general symptoms in case of hypocorticism than normocorticism, and of them significantly higher rate of hypocorticoid than normocorticoid cows died or were emergency slaughtered due to mastitis. No similar tendency was found, however in cows with GP mastitis (*Table 12*), or in mastitis occurring in the later postpartum periods (details are not given).

Table 12: The effect of hypocorticism on the course of mastitis caused by various pathogens on day 1-14 after calving (*Exp. 2*).

Mastitis pathogen	Adrenocortical function*	All (n)	Of them: died + emergency slaughtered	Clinical symptoms at the outbreak (x ± SD) Rectal temperature	Score of systemic signs	altogether
GP:	Normocorticism	33	2 (6.1 %)	39.7 ± 0.6	1.3 ± 0.6	5.2 ± 1.3
	Hypocorticism	7	1 (14.3 %)	39.8 ± 1.0	1.6 ± 1.0	5.3 ± 2.0
	P<		ns	ns	ns	ns
GN + NDP:	Normocorticism	20	2 (10.0 %)	40.3 ± 0.7	2.5 ± 0.5	7.1 ± 1.4
	Hypocorticism	10	6 (60.0 %)	41.3 ± 0.4	3.0 ± 0.0	8.1 ± 1.0
	P<		*0.05*	*0.001*	*0.001*	*0.05*

Notes: *Based on the cortisol response after a standard low dose ACTH challenge
GN: Gram-negative; GP: Gram-positive; NDP mastitis: mastitis with no detected pathogens

Table 13: Mastitis pathogens isolated and identified in *Exp. 3*.

	Mastitis diagnosed as a single disease		
	In early puerperium (day 0 to 14)	In late puerperium (day 15 to 28)	During the peak lactation (day 29 to 60)
S. aureus	2	1	1
Coagulase-negative Staphylococci (CNS)	6	--	2
Strep. dysgalactiae	--	1	2
Strep. uberis	3	2	3
Other Str.	--	--	--
E. coli	7 (of them died: 1)	3	4
Klebsiella	--	1	1
Pseudomonas aeruginosa	--	--	1
No detected pathogen (NDP)	6 (of them died: 2)	3	6
All	24	11	20

4.2.3.4. Mastitis-related endocrine and metabolic alterations in Exp. 3

In the early and late puerperium and during the peak lactation 24, 11 and 20 mastitic cows and their 13, 10 and 14 healthy counterparts were involved in the study, respectively. The pathogens isolated and identified are shown in *Table 13*. One and two cows with *E coli* and NDP mastitis died due to this disease on the 2nd to 3rd day of the course. All these cases lost were diagnosed in the early puerperal phase. In all the others the general symptoms disappeared within 1 to 3 days after the outbreak, which was followed by complete clinical recovery in most of the affected quarters soon (*Table 13*). The endocrine and metabolic pattern of cows with GN mastitis was very close to those observed in cows with NDP mastitis (details

are not given). So their data were pooled and compared to corresponding findings in cows with GP mastitis as well as in healthy individuals.

Compared to the healthy controls and those with GP intramammary infection the cows with GN+NDP mastitis were characterized by significantly elevated *cortisol* levels at the beginning of the sampling procedure (e.g. in samples taken at 14.00 h on the day of diagnosis) representing the first about 7-14 h of the clinical course. Although the individual differences were quite obvious, in the subsequent hours a sharp drop was seen in cows with GN+NDP mastitis, while the cortisol level of healthies and of those with GP mastitis remained unchanged. So the between-group differences disappeared within about 16 h (*Table 14*). These time-related changes in cortisol could be observed in almost all the GN+NDP mastitic cases, regardless of the stage of lactation when mastitis was diagnosed. No similar tendency was seen, however in animals died soon (*Fig. 7*).

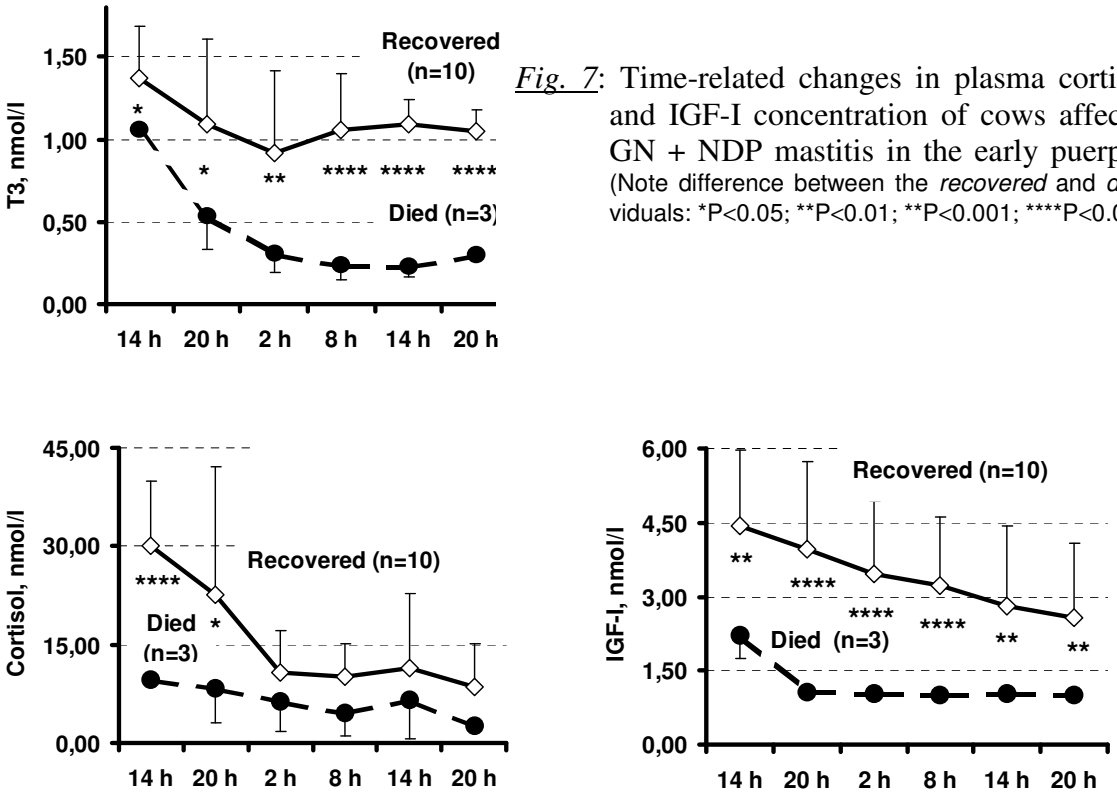


Fig. 7: Time-related changes in plasma cortisol, T_3 and IGF-I concentration of cows affected by GN + NDP mastitis in the early puerperium. (Note difference between the *recovered* and *died* individuals: *P<0.05; **P<0.01; **P<0.001; ****P<0.0001)

The circulating level of *thyroid* hormones (T_4 and T_3) was lower in cows with NDP+GN mastitis than in their healthy counterparts even in the first samples, and it continued to decrease in the first about 36 h of the course. Their nadir was seen in the morning samples of the 2nd day. These time-related changes in thyroid hormones were a bit more obvious in T_3 than in T_4, characterizing the cases diagnosed in early puerperium rather than those observed later (*Tables 15* and *16*).

There were no mastitis related differences in IGF-I levels in the first some hours of the course. Later however, a similar but less rapid tendency was observed also in IGF-I as in T_4 and T_3 (*Table 17*). In cows with GP mastitis only a mild depression was seen in both the thyroid hormone and IGF-I concentrations (*Tables 17-17*).

Extremely low levels of thyroid hormones and IGF-I were determined in the 3 cows died of GN mastitis in the first two weeks of lactation (*Fig. 7*).

Table 14: Mastitis-related alterations in *cortisol* levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
Cortisol, nmol/l									
Early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	$25,38^{ab+}$	$19,30^{a+}$	9,79	8,78	10,34	**7,61**	*5.76*	*9.01*
(n=13)	±SD	12,31	18,12	6,16	5,22	10,36	6,29		
GP mastitis	x	$11,08^{a}$	9,66	10,95	8,96	8,23	8,94	*0.34*	*ns*
(n=11)	±SD	6,39	7,58	10,96	3,37	4,15	4,05		
Healthy control	x	$9,04^{b}$	$7,64^{a}$	7,97	8,18	7,80	7,37	*0.36*	*ns*
(n=13)	±SD	4,25	3,19	3,52	3,47	3,37	3,06		
F=		*14.09*	*3.65*	*0.51*	*0.12*	*0.50*	*0.38*		
$LSD_{P<0.05}$		*7.38*	*10.17*	*ns*	*ns*	*ns*	*ns*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	$24,51^{ab+}$	$19,13^{ab+}$	8,91	9,81	**7,91**	8,67	*10.26*	*6.70*
(n=7)	±SD	9,99	5,67	4,72	5,08	2,99	2,95		
GP mastitis	x	$10,83^{a}$	$10,60^{a}$	10,70	10,93	12,61	10,56	*0.10*	*ns*
(n=4)	±SD	5,34	4,65	2,24	4,50	6,18	5,76		
Healthy control	x	$9,21^{b}$	$8,87^{b}$	8,76	8,76	8,58	8,64	*0.04*	*ns*
(n=10)	±SD	4,04	3,17	3,79	3,52	3,57	3,39		
F=		*11.20*	*11.72*	*0.34*	*0.39*	*1.97*	*0.49*		
$LSD_{P<0.05}$		*8.10*	*5.24*	*ns*	*ns*	*ns*	*ns*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	$18,15^{ab+}$	$15,50^{+}$	11,30	9,66	6,74	**6,34**	*3.18*	*8.13*
(n=12)	±SD	11,38	15,01	9,11	6,81	3,33	3,86		
GP mastitis	x	$8,51^{a}$	7,80	8,89	7,01	7,58	8,09	*0.21*	*ns*
(n=8)	±SD	3,47	3,82	5,08	4,40	3,97	3,97		
Healthy control	x	$8,86^{b}$	8,92	9,13	8,85	8,81	8,82	*0.04*	*ns*
(n=14)	±SD	3,15	3,17	3,99	3,57	3,61	3,62		
F=		*6.52*	*2.20*	*0.48*	*0.65*	*1.09*	*1.42*		
$LSD_{P<0.05}$		*6.68*	*ns*	*ns*	*ns*	*ns*	*ns*		
In all evaluated cows									
NDP+GN mastitis	x	$22,48^{ab+}$	$17,84^{ab+}$	10,16	9,33	8,46	**7,36**	*14.13*	*4.96*
(n=32)	±SD	11,65	14,72	7,02	5,67	7,06	4,75		
GP mastitis	x	$10,14^{a}$	$9,15^{a}$	10,19	8,62	8,77	8,93	*0.37*	*ns*
(n=23)	±SD	5,27	5,81	8,03	4,01	4,63	4,21		
Healthy control	x	$9,02^{b}$	$8,46^{b}$	8,62	8,59	8,39	8,26	*0.21*	*ns*
(n=37)	±SD	3,70	3,15	3,70	3,43	3,45	3,34		
F=		*29.84*	*9.91*	*0.69*	*0.28*	*0.04*	*1.02*		
$LSD_{P<0.05}$		*4.30*	*5.20*	*ns*	*ns*	*ns*	*ns*		

Notes: $^{+}$Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

^{ab}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 15: Mastitis-related alterations in the thyroxin (T_4) levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
Thyroxin (T_4), nmol/l									
Early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	**38,5**	31,0^{ab}	27,0^{ab+}	26,2^{ab+}	26,4^{ab+}	27,0^{ab+}	*2.11*	*10.1*
(n=13)	±SD	9,8	14,5	14,8	12,1	10,9	8,2		
GP mastitis	x	45,2	44,6^{a}	40,6^{a}	39,3^{a}	40,1^{a}	40,2^{a}	*0.45*	*ns*
(n=11)	±SD	11,7	12,4	13,7	11,4	12,6	13,3		
Healthy control	x	46,9	47,4^{b}	44,2^{b}	44,8^{b}	45,5^{b}	46,3^{b}	*0.17*	*ns*
(n=13)	±SD	10,1	10,2	11,0	11,2	11,2	11,8		
F=		*4.15*	*6.32*	*6.04*	*8.80*	*9.42*	*10.08*		
$LSD_{P<0.05}$		*ns*	*10.9*	*11.5*	*10.1*	*10.0*	*9.7*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	46,4^{a}	43,0^{a}	39,4^{a}	35,7^{a}	37,4^{a}	38,2^{a}	*0.40*	*ns*
(n=7)	±SD	17,0	17,4	16,0	15,3	16,1	17,8		
GP mastitis	x	55,6	51,1	49,3	50,4	49,7	52,2	*0.91*	*ns*
(n=4)	±SD	5,7	7,4	5,1	1,2	2,7	4,3		
Healthy control	x	63,2^{a}	64,3^{a}	62,6^{a}	63,0^{a}	63,9^{a}	64,6^{a}	*0.05*	*ns*
(n=10)	±SD	11,4	11,4	12,7	11,6	11,6	11,5		
F=		*3.49*	*5.52*	*6.64*	*10.56*	*9.45*	*8.46*		
$LSD_{P<0.05}$		*15.4*	*15.8*	*15.6*	*14.4*	*14.8*	*15.5*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	55,3	55,1	51,7	47,0^{a}	50,8	50,5^{a}	*0.29*	*ns*
(n=12)	±SD	19,2	18,3	20,4	19,2	22,3	20,6		
GP mastitis	x	62,9	61,5	62,0	60,4	58,6	59,0	*0.05*	*ns*
(n=8)	±SD	25,5	25,0	21,2	21,9	20,2	21,1		
Healthy control	x	66,5	67,3	64,9	65,9^{a}	67,0	68,0^{a}	*0.12*	*ns*
(n=14)	±SD	11,7	12,1	11,6	11,9	12,2	12,6		
F=		*1.23*	*1.50*	*1.94*	*3.97*	*2.57*	*3.12*		
$LSD_{P<0.05}$		*ns*	*ns*	*ns*	*15.9*	*ns*	*16.4*		
In all evaluated cows									
NDP+GN mastitis	x	**46,5**^{a}	42,7^{a}	39,0^{ab}	36,1^{ab+}	38,0^{ab}	38,6^{ab}	*1.29*	*10.2*
(n=32)	±SD	16,7	19,4	20,1	17,9	19,8	18,8		
GP mastitis	x	53,1	51,6	49,6^{a}	48,6^{a}	48,2^{a}	48,8^{a}	*0.28*	*ns*
(n=23)	±SD	18,5	18,3	18,1	17,5	16,6	17,4		
Healthy control	x	58,7^{a}	59,5^{a}	57,0^{b}	57,7^{b}	58,6^{b}	59,5^{b}	*0.17*	*ns*
(n=37)	±SD	14,0	14,3	14,9	14,8	15,0	15,3		
F=		*4.89*	*8.16*	*8.95*	*14.53*	*12.30*	*12.59*		
$LSD_{P<0.05}$		*9.00*	*9.6*	*9.8*	*9.2*	*9.6*	*9.5*		

Notes: $^{+}$Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

^{ab}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 16: Mastitis-related alterations in the 3,3',5-triiodo-thyronine (T_3) levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
3,3',5-triiodo-thyronine (T_3), nmol/l									
Early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	**1,30**	$0,97^{ab}$	$0,78^{ab+}$	$0,86^{ab+}$	$0,89^{ab+}$	$0,92^{ab+}$	*2.34*	*0.36*
(n=13)	±SD	0,30	0,51	0,51	0,47	0,40	0,32		
GP mastitis	x	1,43	$1,39^{a}$	$1,37^{a}$	$1,41^{a}$	$1,43^{a}$	$1,45^{a}$	*0.08*	*ns*
(n=11)	±SD	0,25	0,24	0,26	0,38	0,47	0,42		
Healthy control	x	1,52	$1,53^{b}$	$1,41^{b}$	$1,42^{b}$	$1,46^{b}$	$1,50^{b}$	*0.36*	*ns*
(n=13)	±SD	0,28	0,28	0,31	0,28	0,33	0,37		
F=		*2.04*	*8.03*	*10.87*	*8.78*	*8.18*	*9.65*		
$LSD_{P<0.05}$		*ns*	*0.32*	*0.33*	*0.34*	*0.35*	*0.32*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	$1,32^{ab}$	$1,18^{ab}$	$1,04^{ab}$	$0,95^{ab}$	$1,05^{ab}$	$0,99^{ab}$	*0.75*	*ns*
(n=7)	±SD	0,39	0,40	0,39	0,39	0,48	0,46		
GP mastitis	x	$1,75^{a}$	$1,67^{a}$	$1,60^{a}$	$1,62^{a}$	$1,66^{a}$	$1,63^{a}$	*0.11*	*ns*
(n=4)	±SD	0,35	0,30	0,30	0,33	0,32	0,34		
Healthy control	x	$1,78^{b}$	$1,82^{b}$	$1,71^{b}$	$1,78^{b}$	$1,80^{b}$	$1,84^{b}$	*0.18*	*ns*
(n=10)	±SD	0,33	0,34	0,32	0,32	0,32	0,30		
F=		*3.80*	*6.85*	*8.29*	*12.26*	*8.30*	*11.27*		
$LSD_{P<0.05}$		*0.42*	*0.42*	*0.41*	*0.41*	*0.45*	*0,44*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	1,73	1,59	$1,46^{a}$	$1,47^{ab}$	$1,50^{a}$	$1,50^{a}$	*0.33*	*ns*
(n=12)	±SD	0,52	0,54	0,63	0,63	0,68	0,72		
GP mastitis	x	2,04	1,97	1,94	$1,99^{a}$	1,95	1,99	*0.03*	*ns*
(n=8)	±SD	0,68	0,65	0,53	0,56	0,44	0,51		
Healthy control	x	2,05	2,08	$2,00^{a}$	$2,03^{b}$	$2,09^{a}$	$2,11^{a}$	*0.09*	*ns*
(n=14)	±SD	0,48	0,51	0,51	0,52	0,52	0,54		
F=		*1.31*	*2.65*	*3.36*	*3.57*	*3.67*	*3.55*		
$LSD_{P<0.05}$		*ns*	*ns*	*0.51*	*0.52*	*0.52*	*0.56*		
In all evaluated cows									
NDP+GN mastitis	x	**1,46**^{a}	$1,25^{ab}$	$1,09^{ab+}$	$1,11^{ab+}$	$1,15^{ab+}$	$1,16^{ab+}$	*1.90*	*0.30*
(n=32)	±SD	0,45	0,56	0,60	0,58	0,59	0,59		
GP mastitis	x	1,70	$1,64^{a}$	$1,61^{a}$	$1,65^{a}$	$1,65^{a}$	$1,67^{a}$	*0.09*	*ns*
(n=23)	±SD	0,52	0,49	0,45	0,50	0,48	0,49		
Healthy control	x	$1,79^{a}$	$1,82^{b}$	$1,71^{b}$	$1,75^{b}$	$1,79^{b}$	$1,82^{b}$	*0.31*	*ns*
(n=37)	±SD	0,43	0,46	0,47	0,47	0,49	0,49		
F=		*4.57*	*11.21*	*13.60*	*14.34*	*13.53*	*14.21*		
$LSD_{P<0.05}$		*0.26*	*0.28*	*0.29*	*0.29*	*0.29*	*0.29*		

Notes: $^{+}$Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

^{ab}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 17: Mastitis-related alterations in the insulin-like growth factor-I (IGF-I) levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
Insulin-like growth factor-I (IGF-I), nmol/l									
Early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	**4,03**	3,39	$2,92^{ab}$	$2,72^{ab}$	$2,42^{ab+}$	$2,42^{ab+}$	*1.72*	*1.46*
(n=13)	±SD	1,67	2,06	1,74	1,63	1,66	1,53		
GP mastitis	x	4,57	4,37	$4,41^{a}$	$4,32^{a}$	$4,28^{a}$	$4,33^{a}$	*0.04*	*ns*
(n=11)	±SD	1,92	1,80	1,86	1,67	1,66	1,40		
Healthy control	x	4,40	4,39	$4,39^{b}$	$4,40^{b}$	$4,38^{b}$	$4,41^{b}$	*0.00*	*ns*
(n=13)	±SD	1,16	1,18	1,14	1,17	1,18	1,15		
F=		*0.37*	*1.41*	*3.63*	*5.09*	*6.81*	*8.62*		
$LSD_{P<0.05}$		*ns*	*ns*	*1.39*	*1.30*	*1.31*	*1.19*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	4,97	4,40	3,94	3,75	3,67	3,66	*0.48*	*ns*
(n=7)	±SD	2,04	1,91	1,91	2,00	2,07	2,09		
GP mastitis	x	5,75	5,74	5,67	5,63	5,61	5,62	*0.00*	*ns*
(n=4)	±SD	1,98	2,16	2,11	2,10	2,10	2,13		
Healthy control	x	5,80	5,79	5,78	5,77	5,78	5,82	*0.00*	*ns*
(n=10)	±SD	1,56	1,55	1,53	1,58	1,59	1,58		
F=		*0.48*	*1.38*	*2.45*	*2.77*	*2.91*	*3.00*		
$LSD_{P<0.05}$		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	7,12	6,26	5,82	$5,64^{a}$	$5,59^{a}$	$5,51^{a}$	*0.61*	*ns*
(n=12)	±SD	2,19	2,75	2,90	2,85	2,82	2,82		
GP mastitis	x	7,17	6,92	6,80	6,67	6,75	6,70	*0.07*	*ns*
(n=8)	±SD	1,79	1,83	2,08	2,01	2,00	1,86		
Healthy control	x	7,66	7,64	7,62	$7,67^{a}$	$7,66^{a}$	$7,65^{a}$	*0.00*	*ns*
(n=14)	±SD	1,49	1,47	1,44	1,55	1,46	1,49		
F=		*0.33*	*1.42*	*2.28*	*2,78*	*3.00*	*3.21*		
$LSD_{P<0.05}$		*ns*	*ns*	*ns*	*2.01*	*1.97*	*1.97*		
In all evaluated cows									
NDP+GN mastitis	x	**5,35**	$4,65^{a}$	$4,22^{a}$	$4,03^{ab}$	$3,88^{ab+}$	$3,85^{ab+}$	*1.67*	*1.37*
(n=32)	±SD	2,38	2,60	2,55	2,52	2,58	2,58		
GP mastitis	x	5,64	5,44	5,42	$5,30^{a}$	$5,27^{a}$	$5,35^{a}$	*0.09*	*ns*
(n=23)	±SD	2,09	2,11	2,14	2,06	2,10	2,01		
Healthy control	x	6,01	$6,00^{a}$	$5,99^{a}$	$6,01^{b}$	$6,00^{b}$	$6,02^{b}$	*0.00*	*ns*
(n=37)	±SD	1,97	1,96	1,94	1,99	1,97	1,96		
F=		*0.82*	*3.13*	*5.57*	*6.99*	*7.86*	*8.49*		
$LSD_{P<0.05}$		*ns*	*1.25*	*1.24*	*1.23*	*1.24*	*1.23*		

Notes: [+]Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

[ab]Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 18: Mastitis-related alterations in the insulin levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
Insulin, µIU/l									
Early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	**5,9**	$20,1^{ab+}$	$16,5^{ab+}$	$10,4^{ab}$	$8,6^{ab}$	$7,2^{ab}$	*8.05*	*6.1*
(n=13)	±SD	4,9	9,0	9,4	7,6	6,6	3,5		
GP mastitis	x	3,8	$3,4^{a}$	$3,5^{a}$	$4,2^{a}$	$3,9^{a}$	$3,8^{a}$	*0.21*	*ns*
(n=11)	±SD	1,2	1,4	2,2	3,5	1,8	1,3		
Healthy control	x	3,8	$3,9^{b}$	$3,6^{b}$	$3,7^{b}$	$3,9^{b}$	$4,1^{b}$	*0.22*	*ns*
(n=13)	±SD	1,4	1,4	1,3	1,3	1,3	1,3		
F=		*1.94*	*38.14*	*21.28*	*7.21*	*5.50*	*8.21*		
$LSD_{P<0.05}$		*ns*	*4.8*	*5.0*	*4.3*	*3.6*	*2.0*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	8,9	$15,9^{ab}$	$11,9^{ab}$	$10,5^{ab}$	$10,4^{ab}$	$8,3^{a}$	*0.97*	*ns*
(n=7)	±SD	7,5	9,0	7,9	6,7	7,3	4,7		
GP mastitis	x	3,9	$3,4^{a}$	$3,5^{a}$	$3,8^{a}$	$4,9^{a}$	5,0	*1.54*	*ns*
(n=4)	±SD	1,2	1,3	1,0	0,9	1,2	1,1		
Healthy control	x	4,2	$4,2^{b}$	$4,0^{b}$	$4,2^{b}$	$4,2^{b}$	$4,4^{a}$	*0.13*	*ns*
(n=10)	±SD	1,1	1,1	1,1	1,2	1,1	1,1		
F=		*2.73*	*11.94*	*7.01*	*6.08*	*4.55*	*4.03*		
$LSD_{P<0.05}$		*ns*	*6.3*	*5.4*	*4.7*	*5.1*	*3.4*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	**7,5**	$12,4^{ab+}$	$10,1^{ab}$	$8,8^{ab}$	$8,5^{a}$	$8,4^{a}$	*2.62*	*3.3*
(n=12)	±SD	2,1	6,1	4,7	3,2	2,5	1,8		
GP mastitis	x	6,3	$5,5^{a}$	$5,3^{a}$	$6,0^{a}$	6,5	6,7	*0.32*	*ns*
(n=8)	±SD	2,5	3,0	3,1	2,6	2,6	2,8		
Healthy control	x	6,2	$6,3^{b}$	$6,0^{b}$	$6,2^{b}$	$6,2^{a}$	$6,3^{a}$	*0.04*	*ns*
(n=14)	±SD	2,0	2,0	2,0	2,0	2,0	2,1		
F=		*1.34*	*9.47*	*6.41*	*4.07*	*3.48*	*3.20*		
$LSD_{P<0.05}$		*ns*	*3.8*	*3.1*	*2.4*	*2.1*	*2.0*		
In all evaluated cows									
NDP+GN mastitis	x	**7,2**^{ab}	$16,3^{ab+}$	$13,1^{ab+}$	$9,8^{ab}$	$9,0^{ab}$	$7,9^{ab}$	*9.99*	*3.4*
(n=32)	±SD	4,8	8,5	7,9	5,9	5,5	3,3		
GP mastitis	x	$4,7^{a}$	$4,1^{a}$	$4,1^{a}$	$4,8^{a}$	$5,0^{a}$	$5,0^{a}$	*0.69*	*ns*
(n=23)	±SD	2,1	2,2	2,5	2,9	2,3	2,3		
Healthy control	x	$4,8^{b}$	$4,9^{b}$	$4,6^{b}$	$4,8^{b}$	$4,9^{b}$	$5,0^{b}$	*0.19*	*ns*
(n=37)	±SD	1,9	1,9	1,9	1,9	1,9	1,9		
F=		*5.87*	*51.56*	*31.93*	*16.65*	*12.94*	*13.38*		
$LSD_{P<0.05}$		*1.8*	*2.9*	*2.8*	*2.2*	*2.0*	*1.4*		

Notes: $^{+}$Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

^{ab}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 19: Mastitis-related alterations in the non-esterified fatty acids (NEFA) levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
Non-esterified fatty acids (NEFA), mmol/l									
In the early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	0,59	0,72^{ab+}	0,66^{a}	0,57	0,55	**0,51**	*1.58*	*0.19*
(n=13)	±SD	0,23	0,25	0,24	0,20	0,20	0,20		
GP mastitis	x	0,48	0,48^{a}	0,49	0,48	0,46	0,44	*0.07*	*ns*
(n=11)	±SD	0,17	0,17	0,21	0,18	0,19	0,18		
Healthy control	x	0,46	0,46^{b}	0,46^{a}	0,44	0,41	0,39	*0.29*	*ns*
(n=13)	±SD	0,15	0,16	0,14	0,13	0,11	0,10		
F=		*1.79*	*6.73*	*3.70*	*1.94*	*2.25*	*1.74*		
$LSD_{P<0.05}$		*ns*	*0.17*	*0.18*	*ns*	*ns*	*ns*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	0,39	0,44^{a}	0,46	0,42	0,38	0,37	*0.72*	*ns*
(n=7)	±SD	0,08	0,11	0,13	0,14	0,10	0,10		
GP mastitis	x	0,39	0,43	0,44	0,39	0,33	0,31	*0.82*	*ns*
(n=4)	±SD	0,09	0,17	0,16	0,07	0,10	0,11		
Healthy control	x	0,33	0,32^{a}	0,35	0,32	0,31	0,30	*0.97*	*ns*
(n=10)	±SD	0,05	0,06	0,06	0,06	0,05	0,05		
F=		*1.99*	*3.34*	*2.41*	*2.40*	*1.63*	*1.62*		
$LSD_{P<0.05}$		*ns*	*0.12*	*ns*	*ns*	*ns*	*ns*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	0,37^{a}	0,40^{a}	0,42^{a}	0,38	0,34	0,32	*0.65*	*ns*
(n=12)	±SD	0,13	0,16	0,21	0,20	0,12	0,11		
GP mastitis	x	0,37^{b}	0,36^{b}	0,38	0,35	0,33	0,31	*0.28*	*ns*
(n=8)	±SD	0,15	0,14	0,14	0,13	0,13	0,14		
Healthy control	x	0,26^{ab}	0,26^{ab}	0,27^{a}	0,26	0,26	0,25	*0.16*	*ns*
(n=14)	±SD	0,06	0,05	0,06	0,06	0,06	0,06		
F=		*3.99*	*4.61*	*3.60*	*2.47*	*2.30*	*1.78*		
$LSD_{P<0.05}$		*0.10*	*0.11*	*0.14*	*ns*	*ns*	*ns*		
In all the evaluated cows									
NDP+GN mastitis	x	0,46^{a}	0,54^{ab+}	0,53^{ab+}	0,47^{a}	0,43^{a}	**0,41**^{a}	*2.09*	*0.11*
(n=32)	±SD	0,20	0,24	0,23	0,20	0,18	0,17		
GP mastitis	x	0,43	0,43^{a}	0,44^{a}	0,42	0,39	0,38	*0.56*	*ns*
(n=23)	±SD	0,16	0,16	0,18	0,16	0,17	0,17		
Healthy control	x	0,35^{a}	0,34^{b}	0,36^{b}	0,34^{a}	0,33^{a}	0,31^{a}	*0.84*	*ns*
(n=37)	±SD	0,13	0,13	0,12	0,11	0,10	0,09		
F=		*4.10*	*10.33*	*7.69*	*5.90*	*3.89*	*4.42*		
$LSD_{P<0.05}$		*0.09*	*0.10*	*0.10*	*0.09*	*0.08*	*0.08*		

Notes: $^{+}$Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

^{ab}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 20: Mastitis-related alterations in the βOH-butyrate (BHB) levels in *Exp. 3*.

		Day of outbreak		The subsequent day				Time-related	
		14 h	20 h	2 h	8 h	14 h	20 h	F=	$LSD_{P<0.05}$
βOH-butyrate (BHB), mmol/l									
In the early puerperium (day ≤14 after calving)									
NDP+GN mastitis	x	**1,68**[ab]	1,25[+]	0,88[+]	0,76[+]	0,75[+]	0,70[+]	*10.44*	*0.37*
(n=13)	±SD	0,69	0,61	0,28	0,25	0,25	0,26		
GP mastitis	x	1,11[b]	1,04	0,95	0,80	0,77	0,78	*1.49*	*ns*
(n=11)	±SD	0,48	0,51	0,49	0,30	0,25	0,25		
Healthy control	x	0,96[b]	0,95	1,05	0,98	1,00	0,98	*0.08*	*ns*
(n=13)	±SD	0,40	0,37	0,46	0,48	0,52	0,49		
F=		*6.35*	*1.20*	*0.55*	*1.36*	*1.81*	*2.12*		
$LSD_{P<0.05}$		*0.47*	*ns*	*ns*	*ns*	*ns*	*ns*		
In the late puerperium (day 15-28 after calving)									
NDP+GN mastitis	x	**1,23**	1,13	0,90	0,80	0,75[+]	0,74[+]	*3.19*	*0.36*
(n=7)	±SD	0,47	0,42	0,30	0,16	0,19	0,14		
GP mastitis	x	1,05	0,92	0,88	0,85	0,83	0,79	*0.48*	*ns*
(n=4)	±SD	0,28	0,30	0,28	0,18	0,26	0,26		
Healthy control	x	0,89	0,88	0,95	0,91	0,87	0,89	*0.04*	*ns*
(n=10)	±SD	0,50	0,47	0,48	0,47	0,46	0,43		
F=		*1.13*	*0.73*	*0.06*	*0.20*	*0.23*	*0.44*		
$LSD_{P<0.05}$		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*		
During the peak lactation (day 29-60 after calving)									
NDP+GN mastitis	x	0,93	0,89	0,75	0,72	0,71	0,73	*1.44*	*ns*
(n=12)	±SD	0,44	0,46	0,14	0,13	0,10	0,10		
GP mastitis	x	0,72	0,74	0,69	0,68	0,68	0,64	*0.16*	*ns*
(n=8)	±SD	0,33	0,34	0,22	0,19	0,20	0,15		
Healthy control	x	0,78	0,79	0,84	0,80	0,80	0,78	*0.09*	*ns*
(n=14)	±SD	0,28	0,28	0,29	0,30	0,26	0,26		
F=		*0.98*	*0.45*	*1.17*	*0.76*	*1.10*	*1.35*		
$LSD_{P<0.05}$		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*		
In all the evaluated cows									
NDP+GN mastitis	x	**1,30**[ab]	1,09[+]	0,84[+]	0,76[+]	0,74[+]	0,72[+]	*11.93*	*0.20*
(n=32)	±SD	0,64	0,53	0,25	0,19	0,19	0,18		
GP mastitis	x	**0,96**[a]	0,92	0,84	0,77	0,75	0,73[+]	*1.73*	*0.22*
(n=23)	±SD	0,43	0,43	0,39	0,25	0,23	0,23		
Healthy control	x	0,87 [b]	0,87	0,94	0,89	0,89	0,88	*0,17*	*ns*
(n=37)	±SD	0,37	0,35	0,39	0,39	0,40	0,38		
F=		*6.93*	*2.45*	*0.91*	*1.96*	*2.61*	*2.26*		
$LSD_{P<0.05}$		*0.28*	*ns*	*ns*	*ns*	*ns*	*ns*		

Notes: [+]Within a row the signed group mean is significantly different (P<0.05) from the lowest / highest value (in bold) of the corresponding data set.

[ab]Within a column the group means sharing the same superscripts are significantly different (P<0.05)

At the beginning of the sampling procedure the level of *insulin* was a bit higher in cows with NDP+GN than in those with GP mastitis, as well as in healthy controls. Afterwards further significant, but only temporary increase (forming the peak values in the 2nd and/or 3rd samples) was determined in the NDP+GN mastitic cows, but never in the other two groups of animals. These time-related insulin changes in the NDP+GN mastitic cows were obvious in all 3 stages of lactation studied, but the tendency seemed to be more pronounced in the early puerperium than later (*Table 18*).

Similar (but from statistical point of view less significant) tendencies forming a mild plateau in the 2nd and/or 3rd samples in cows with NDP+GN mastitis only were observed also in plasma levels of *non-esterified fatty acids* (NEFA) (*Table 19*).

In the first samples elevated (≥1.00 mmol/l) *βOH-butyrate* (BHB) concentrations were found in 20 of the 32 cows with NDP+GN mastitis, but only in 7 of the 23 animals with GP mastitis and in 7 of the 37 controls ($P<0.001$). Later a dramatic reduction was seen in rate of hyperketonaemic individuals among the NDP+GN mastitic cows ($P<0.001$), but their proportion decreased hardly or remained unchanged in the other two groups. So at the end of the sampling period this rate was 1/32, 4/23 and 6/37 ($P<0.2$) in cows with NDP+GN or GP mastitis and controls, respectively. These changes in BHB levels characterized mainly the cows studied in the early puerperium (*Table 20*).

The plasma and milk *progesterone* (P_4) concentrations mirrored the *lack of luteal activity* in all of the cows in the early puerperium, as well as in 16 of the 22 and 11 of the 34 animals in the late puerperium and during the peak lactation, respectively. In some of these cows, which might be in the stage of postpartum ovarian acyclicity or in the follicular phase of their cycle, simultaneously with the above detailed mastitis-related cortisol elevation also slight, temporary increase in P_4 levels was observed sometimes. These mild, usually non-luteal P_4 peaks of 1.23 to 3.16 nmol/l in plasma and 1.03 to 1.78 nmol/l in skim milk coincided with the cortisol increase in all the cases, and disappeared completely in the second half of the sampling period. The phenomenon was recorded in 15 of the 22 cows with NDP+GN mastitis, but neither in those with GP mastitis nor in the healthy controls. No similar temporary P_4 elevation was seen in the three cows died some hours later, either. According to the detected P_4 levels in their plasma and milk, 10 of the cows with NDP+GN mastitis were proved to be in the *luteal stage* of their ovarian cycle. In 8 of them the P_4 levels remained elevated till the end of the sampling procedure. However, in the further 2 cows the corpus luteum (CL) related concentrations of P_4 in the first two blood (5.43 – 5.78 and 8.73 – 8.77 nmol/l) and milk samples (3.36 – 3.51 and 3.49 – 3.56 nmol/l) were followed by a sharp decline reaching the minimal levels of 0.95 and 0.78 nmol/l in blood and 0.98 and 0.92 nmol/l in milk at the end of the sampling period. The mastitis outbreak was observed in both of these cows during the peak lactation, but in the first few days (e.g. on days 32 and 37) of this period. No similar P_4 drop was seen in the 6 and 13 cows showing luteal activity in the GP mastitic and healthy groups.

4.2.3.5. Challenging the adrenocortical and thyroid function in mastitic and healthy cows (Exp. 4)

In *Exp. 4* the ACTH-induced cortisol response and TRH-challenged thyroid hormone release were studied in 44, 17 and 35 new cases of mastitis, and in 44, 17 and 35 of their healthy counterparts in the early and late puerperium and during the peak lactation, respectively. The list of isolated and identified pathogens is presented in the *Table 21*. Three of these cows died or were emergency slaughtered due to *S. aureus*, *E. coli*, or NDP mastitis (one in each group), all of them in the early puerperal phase. As seen in *Exp. 3*, in those animals recovered the general symptoms disappeared within 1 to 3 days after the outbreak. However, some of them had to be culled later.

Table 21: Mastitis pathogens isolated and identified in *Exp. 4*.

	Mastitis diagnosed as a single disease		
	In early puerperium (day 0 to 14)	In late puerperium (day 15 to 28)	During the peak lactation (day 29 to 60)
S. aureus	2 (of them died: 1)	1	1
Coagulase-negative Staphylococci (CNS)	5	1	1
Strep. dysgalactiae	2	2	2
Strep. uberis	4	3	4
Other Str.	2	--	3
E. coli	13 (of them died: 1)	4	11
Klebsiella	1	1	1
Pseudomonas aeruginosa	--	1	--
Citrobacter freundii	1	--	--
No detected pathogen (NDP)	14 (of them died: 1)	4	12
All	44	17	35

Table 22: ACTH-induced cortisol response in *Exp. 4*.

	Cortisol, nmol/l (x±SD)		Temporary hypocorticism
	Basal (in t_0 sample)	Stimulated (in t_{60} sample)	
In the early puerperium (day ≤14 after calving)			
NDP+GN mastitis (n=29)♦	$15,87 \pm 11,60^{ab}$	$80,05 \pm 31,97$	4♦
GP mastitis (n=15)♦♦	$10,54 \pm 4,24^{a}$	$88,78 \pm 33,03$	2
Healthy control (n=44)	$11,64 \pm 4,75^{b}$	$85,41 \pm 44,93$	7
F=	*3,48*	*0,29*	
$LSD_{P<0.05}$	*4,51*	*ns*	
In the late puerperium (day 15-28 after calving)			
NDP+GN mastitis (n=10)	$11,12 \pm 8,21$	$103,90 \pm 26,36$	--
GP mastitis (n=7)	$8,05 \pm 4,36$	$99,52 \pm 25,14$	--
Healthy control (n=17)	$10,32 \pm 5,42$	$105,11 \pm 33,47$	--
F=	*0,53*	*0,09*	
$LSD_{P<0.05}$	*n*	*n*	
During the peak lactation (day 29-60 after calving)			
NDP+GN mastitis (n=24)	$12,14 \pm 11,96$	$101,11 \pm 35,01$	2
GP mastitis (n=11)	$12,92 \pm 10,10$	$111,53 \pm 23,72$	--
Healthy control (n=35)	$10,75 \pm 5,33$	$115,20 \pm 41,30$	--
F=	*0,33*	*1,05*	
$LSD_{P<0.05}$	*ns*	*ns*	

Note: ♦Two of these 4 cows died of *E. coli* and NDP mastitis

♦♦One of these cows was emergency slaughtered due to *S. aureus* mastitis. However, that cow showed physiological response to the standard ACTH challenge.

[ab]Within a column the group means sharing the same superscripts are significantly different (P<0.05)

Table 23: ACTH-induced cortisol response in cows with GN+NDP mastitis in the early puerperium (*Exp. 4*)

	Cortisol, nmol/l (x±SD)			Temporary hypocorticism
	Basal (in t_0 sample)	Challenged (in t_{60} sample)	Increment ($t_0 \Rightarrow t_{60}$)	
GN+NDP mastitis, in the early puerperium (day ≤14 after calving)				
Mild (n=11)	$10,00 \pm 6,09^{a}$	$94,79 \pm 26,93$	$84,79 \pm 28,18^{ab}$	0
Moderate (n=9)	$11,53 \pm 5,95^{b}$	$69,60 \pm 31,63$	$58,07 \pm 30,46^{ac}$	2
Severe (n=9)♦	$27,38 \pm 13,10^{ab}$	$72,48 \pm 34,40$	$45,10 \pm 27,60^{bc}$	2♦
F=	*11,17*	*2,04*	*39,57*	
$LSD_{P<0.05}$	*8,70*	*ns*	*11,19*	

Note: ♦Two of these 4 cows died of *E. coli* and NDP mastitis

^{abc}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

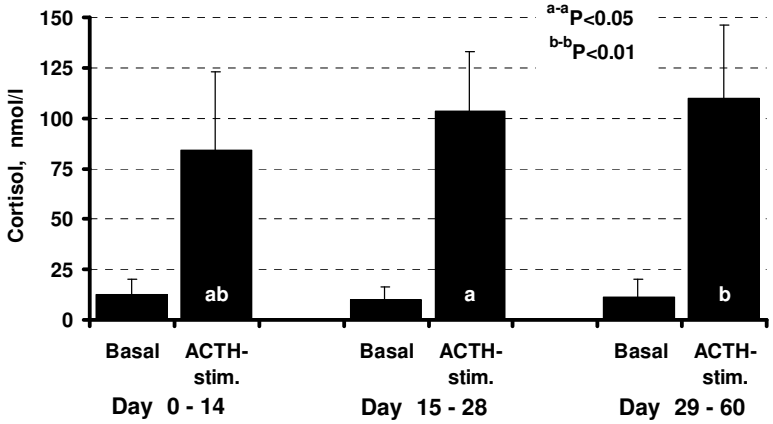


Fig. 8: The basal cortisol levels and ACTH-induced cortisol responses in cows in the early (n=88) and late puerperium (n=34) and during the peak lactation (n=70) (*Exp. 4*)

ANOVA ($^{a}P<0.05$; $^{b}P<0.01$):
Basal: F=1.85, ns;
Challenged: F=10.31,
$LSD_{P<0.05}$ = 10.31 nmol/l,
$LSD_{P<0.01}$ = 14.34 nmol/l

The endocrine response to ACTH and TRH challenge in cows with GN mastitis was almost the same, as in those with NDP mastitis (details are not given). So the data of NDP and GN mastitis affected cows were pooled and compared to those with GP mastitis, as well as of the healthy individuals.

Regardless of the interval elapsed since calving the basal cortisol concentration was quite similar in all three stages of the postpartum period. However, the mean of cortisol response to the standard low dose ACTH challenge was significantly lower in the first two weeks after calving than later (*Fig. 8*). Using similar principles as in *Exp. 2* the lower threshold of physiological cortisol response was calculated to be almost the same (40.48 nmol/l; ≈ 40 nmol/l) as in the earlier experiment. According to this cut-off level temporary hypocorticism was supposed to occur in 13 of 88 cows in the early puerperium, but only in 2 of 104 animals (χ^2=9.457; P<0.01) sampled later.

In the *early puerperium* the cows with NDP+GN mastitis were characterized by higher cortisol levels in their basal (t_0) samples than those with GP mastitis, as well as their healthy counterparts. The standard low dose ACTH challenge induced (further) elevation in mean values of all three groups (*Table 22*). In cows with NDP+GN mastitis the increase in baseline level was more obvious, but the subsequent cortisol increment was less pronounced in severe than in moderate and mild cases (*Table 23*). In this form of mastitis 4 cows showed lower than usual cortisol response to the ACTH challenge, and two of them died (*Table 22*). No similar interrelations were observed, however, in cases of GP mastitis (no details are given). In the *late puerperium* and during the *peak lactation* no mastitis related changes were seen in the adrenocortical function (*Table 22*).

The cows were characterized by significantly lower basal T_4 and T_3 concentration in the early than late puerperium and during the peak lactation (*Fig. 9*). An inverse tendency was seen in the

Fig. 9: The basal T_4 and T_3 levels and TRH-induced thyroid hormone responses in cows in the early (n=88) and late puerperium (n=34) and during the peak lactation (n=70) (*Exp. 4*)
ANOVA ($^{a}P<0.05$; $^{b}P<0.01$; $^{cd}P<0.001$):

T_4 (nmol/l), *basal* (0^{th} min.): F=32.60, $LSD_{P<0.05}$ = 6.5, $LSD_{P<0.01}$ = 9.1, $LSD_{P<0.001}$ = 12.7
challenged:, 240^{th} min.: F=19.42, $LSD_{P<0.05}$ = 7.2, $LSD_{P<0.01}$ = 10.1, $LSD_{P<0.001}$ = 14.1
challenged:, 360^{th} min.: F=20.89, $LSD_{P<0.05}$ = 7.3, $LSD_{P<0.01}$ = 10.2, $LSD_{P<0.001}$ = 14.3
T_3 (nmol/l), *basal* (0^{th} min.): F=23.20, $LSD_{P<0.05}$ = 0.21, $LSD_{P<0.01}$ = 0.29, $LSD_{P<0.001}$ = 0.40
challenged:, 240^{th} min.: F=21.48, $LSD_{P<0.05}$ = 0.25, $LSD_{P<0.01}$ = 0.35, $LSD_{P<0.001}$ = 0.49
challenged:, 360^{th} min.: F=17.38, $LSD_{P<0.05}$ = 0.29, $LSD_{P<0.01}$ = 0.40, $LSD_{P<0.001}$ = 0.56

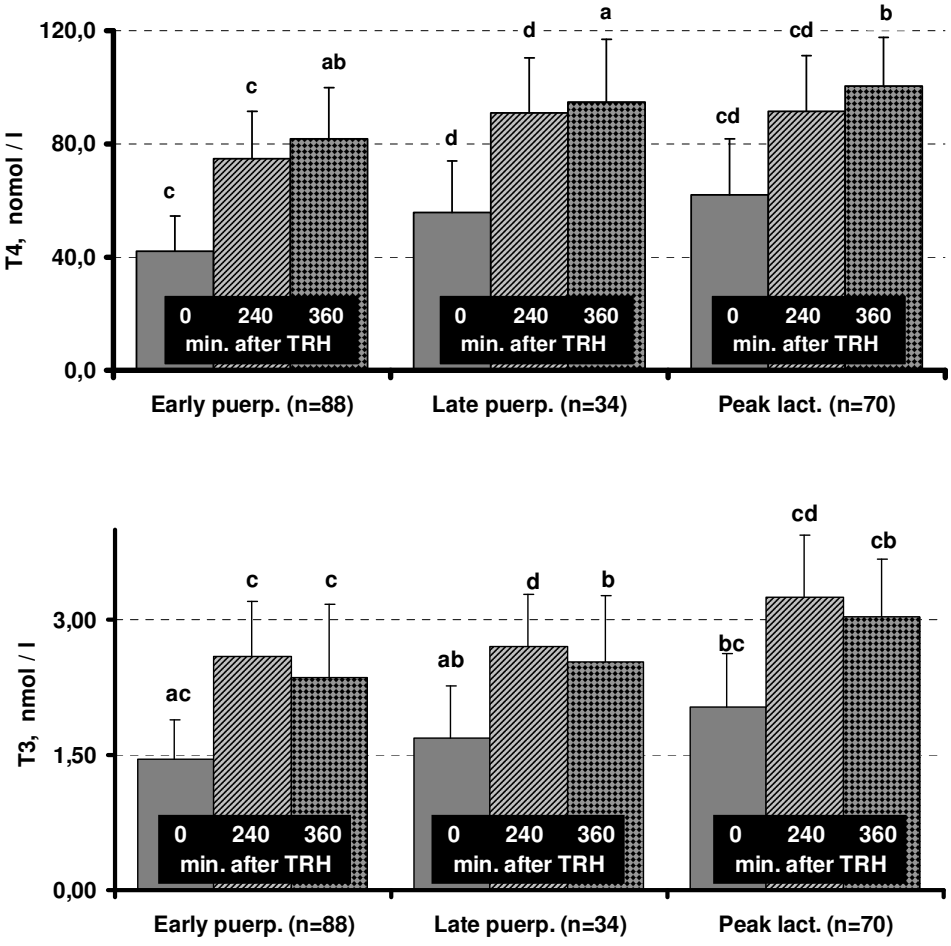
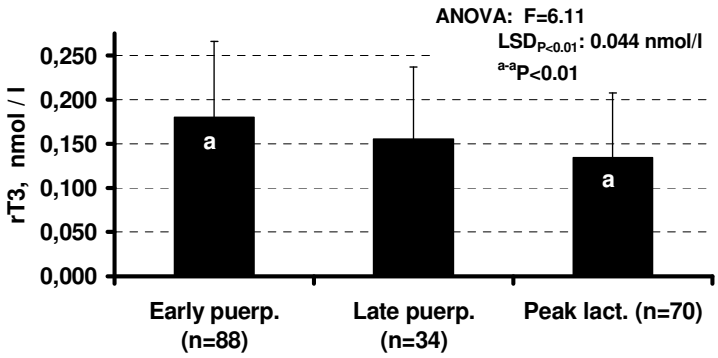


Fig. 10: The basal rT_3 levels in cows in the early (n=88) and late puerperium (n=34) and during the peak lactation (n=70) (*Exp. 4*)



circulating levels of rT_3 (*Fig. 10*). Also the TRH-induced thyroid hormone responses were more obvious in the advanced stages of lactation than in the early postpartum period (*Fig. 9*).

Comparing to those found in healthy controls and in cows with GP mastitis the basal T_4 and T_3 levels diminished in the cases of NDP+GN mastitis. Although the standard dose TRH challenge could induce further T_4 and T_3 release in all animals, this thyroid hormone response was more pronounced in healthies and in cows with GP mastitis than in those with NDP+GN mastitis (*Table 24*). These mastitis-related differences in thyroid function were the most obvious in the first two weeks after calving mastitis (*Table 24*), and derived mainly from data of cows showing the most severe form of this disease (including those 2 cows died soon after sampling), rather than from cases with only mild or moderate clinical symptoms (*Figs 11* and *12*).

Table 24: The TRH-induced T_4 and T_3 responses and the basal rT3 concentration of healthy cows, as well as of those with NDP+GN mastitis or GP mastitis (*Exp. 4*)

		T_4 (nmol/l)			T_3 (nmol/l)			rT_3 (nmol/l)
		Basal	Stimulated		Basal	Challenged		Basal
		(in t_0 sample)	(in t_{240} sample)	(in t_{360} sample)	(in t_0 sample)	(in t_{240} sample)	(in t_{360} sample)	(in t_0 sample)
In the early puerperium (day ≤14 after calving)								
NDP+GN mastitis♦	x	$35,4^{ab}$	$64,1^{ab}$	$68,3^{ab}$	$1,29^{a}$	$2,32^{ab}$	$1,97^{ab}$	$0,219^{ab}$
(n=29)	±SD	9,9	21,3	23,7	0,47	0,77	0,85	0,100
GP mastitis♦♦	x	$42,3^{a}$	$81,0^{a}$	$88,3^{a}$	1,49	$2,75^{a}$	$2,59^{a}$	$0,149^{a}$
(n=15)	±SD	12,2	11,1	12,8	0,30	0,47	0,47	0,050
Healthy control	x	$46,5^{b}$	$79,8^{b}$	$88,4^{b}$	$1,56^{a}$	$2,72^{b}$	$2,54^{b}$	$0,165^{b}$
(n=44)	±SD	14,2	15,4	16,4	0,45	0,56	0,88	0,089
F=		*6,77*	*8,68*	*11,14*	*3,40*	*4,15*	*4,98*	*4,44*
$LSD_{P<0.05}$		*7,4*	*10,0*	*11,0*	*0,26*	*0,37*	*0,48*	*0,052*
In the late puerperium (day 15-28 after calving)								
NDP+GN mastitis	x	$42,3^{a}$	$77,4^{a}$	$78,7^{ab}$	$1,26^{ab}$	$2,28^{ab}$	$1,91^{ab}$	$0,219^{ab}$
(n=10)	±SD	18,0	23,8	21,7	0,43	0,43	0,65	0,125
GP mastitis	x	57,1	95,1	$99,9^{a}$	$1,83^{a}$	$2,90^{a}$	$2,82^{a}$	$0,137^{a}$
(n=7)	±SD	12,6	15,7	16,9	0,45	0,33	0,54	0,059
Healthy control	x	$63,2^{a}$	$97,4^{a}$	$102,3^{b}$	$1,89^{b}$	$2,88^{b}$	$2,78^{b}$	$0,125^{b}$
(n=17)	±SD	20,5	18,3	24,5	0,71	0,75	0,86	0,066
F=		*4,04*	*3,48*	*3,72*	*3,78*	*3,55*	*4,61*	*3,92*
$LSD_{P<0.05}$		*17,4*	*18,4*	*21,0*	*0,56*	*0,57*	*0,70*	*0,081*
During the peak lactation (day 29-60 after calving)								
NDP+GN mastitis	x	$52,9^{a}$	$80,6^{a}$	$92,7^{ab}$	$1,72^{a}$	$2,91^{ab}$	$2,64^{ab}$	$0,171^{ab}$
(n=24)	±SD	15,8	23,2	22,0	0,63	0,84	0,78	0,092
GP mastitis	x	64,3	93,6	$104,8^{a}$	2,16	$3,46^{a}$	$3,20^{a}$	$0,111^{a}$
(n=11)	±SD	20,8	10,9	9,8	0,55	0,62	0,44	0,076
Healthy control	x	$67,5^{a}$	$98,2^{a}$	$104,4^{b}$	$2,21^{a}$	$3,40^{b}$	$3,26^{b}$	$0,117^{b}$
(n=35)	±SD	22,2	20,3	16,3	0,59	0,61	0,59	0,059
F=		*3,32*	*5,42*	*3,52*	*3,7*	*3,77*	*7,02*	*4,41*
$LSD_{P<0.05}$		*13,2*	*13,4*	*11,7*	*0,46*	*0,49*	*0,43*	*0,049*

Note: ♦Two of these 4 cows died of *E. coli* and NDP mastitis

♦♦One of these cows was emergency slaughtered due to *S. aureus* mastitis. However, that cow showed physiological response to the standard ACTH challenge.

^{ab}Within a column the group means sharing the same superscripts are significantly different (P<0.05)

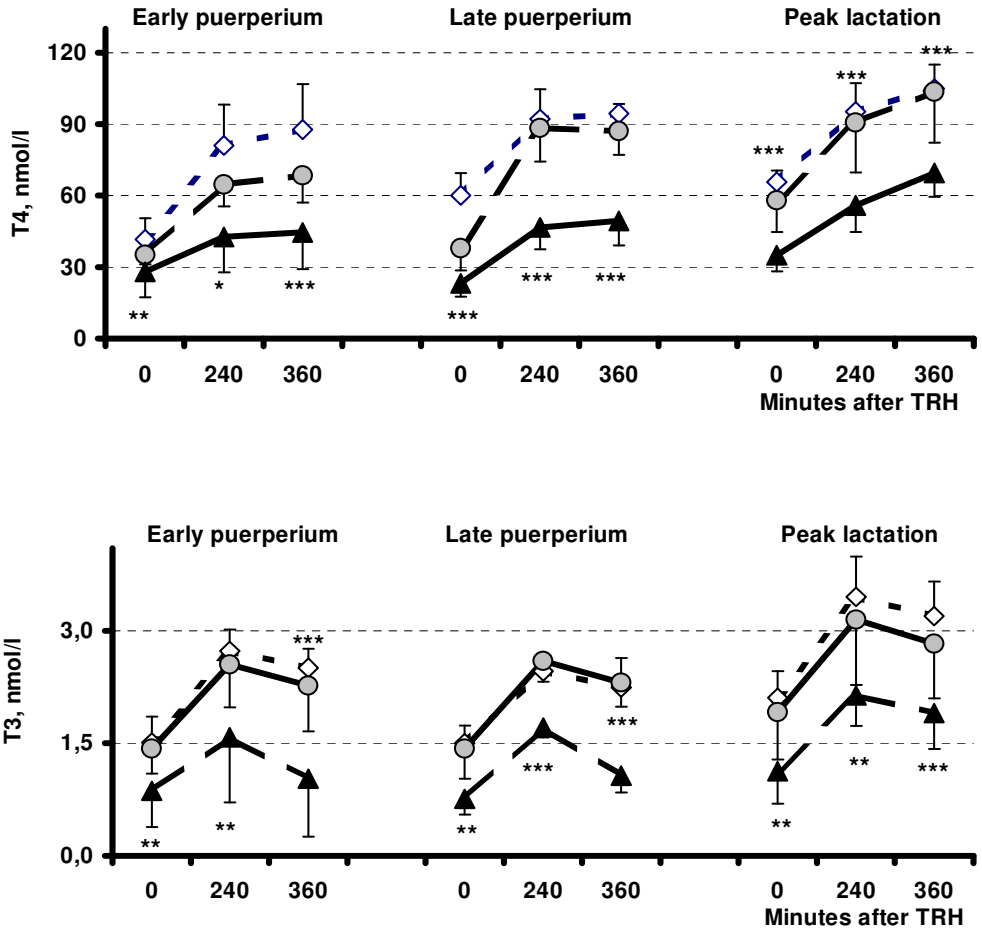


Fig. 11: The TRH-induced T_4 and T_3 responses in cows with mild, moderate or severe forms of NDP+GN mastitis (*Exp. 4*) (Compared with data of cows with NDP and GN mastitis: *P<0.05 **P<0.01 ***P<0.001)

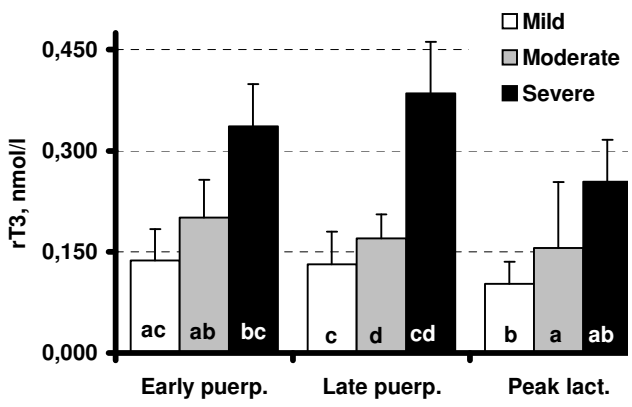


Fig. 12: The basal rT3 levels in cows with mild, moderate or severe forms of NDP+GN mastitis (*Exp. 4*)

ANOVA: Within the corresponding data sets: $^{a}P<0.05$; $^{b}P<0.01$; $^{cd}P<0.001$

Early puerp.: F=33.35, $LSD_{P<0.05}$ = 0.054 nmol/l

Late puerp.: F=19.69, $LSD_{P<0.05}$ = 0.093 nmol/l

Peak lact.: F=9.80, $LSD_{P<0.05}$ = 0.075 nmol/l

4.2.4. DISCUSSION

4.2.4.1. General considerations of this trial

Experimental conditions. Design. Evaluation

In the current trial we wanted to investigate the clinical relevance of *metabolic predisposition* for mastitis caused by various pathogens, as well as of the possible *mastitis-induced endocrine and metabolic alterations in postpartum dairy cows* kept in commercial dairy herds. Concerning the metabolic aspects the supposed consequences of negative energy balance (NEB) were studied. Wide range of deficiency conditions (β-carotene, vitamins A and E, as well as selenium, zinc and copper), as other nutrition related factors have been reported to predispose cows for mastitis, and/or have been found to influence the course of this disease (Ali-Vehmas et al., 1997; Batra et al., 1992; Chew, 1987 and 1993; Harmon and Torre, 1994; Harmon et al., 1994; Kincaid et al., 1984; Scherf et al., 1994; Schukken et al., 1993a). However, studying their effects on mastitis was not the subject of the current research activity. On the other hand certain GN mastitis pathogens (*E. coli* and others) are known to result in a massive intramammary endotoxin loading of the host (Sandholm and Pyörälä, 1995a). Endotoxin mediated diseases and/or the experimental (intravenous or intramammary) administration of LPS have been verified to induce several sorts of endocrine changes: impairments in production and metabolism of thyroid hormones, as well as the involvement of ACTH / cortisol and growth hormone (GH) / IGF-I axes and insulin were observed (detailed below). Beyond these endocrine alterations endotoxin induced temporary increase have been reported to occur in circulating levels of *prolactin* (in lab rodents, humans and ruminants, but not in pig), *glucagon* (in lab rodents and humans) and *catecholamines* (in humans, dogs and adult rodents, but not in newborn and 20-day-old rats) (reviewed by Berczi, 1993). Furthermore the LPS administration caused marked parallel increases in mRNS abundance (both in rodents and pig) and serum level of *leptin* (in rodents only, but not in pig) (Houseknecht et al., 1998, Leininger et al., 2000). However, none of these latter 4 hormones were studied within the framework of the current trial.

This study was intended to spread over almost all sorts of intramammary infections including the predominant contagious microbe of *S. aureus*, as well as the most important GP and GN environmental pathogens. The occurrence rate of GN mastitis is about 15 % in Western Europe (Sandholm and Pyörälä, 1995a), and it was reported to vary between 5 and 15 % in different surveys conducted in Hungary (Gacs et al., 1993; Markus, 2000). This prevalence would have been low for our purposes. However, in the early weeks of lactation GN bacteria may be the predominant mastitis pathogens on farms producing low (<150,000 - 250,000 / ml) somatic cell count milk (LSCCM) (Beaudeau et al., 2002; Green et al., 1996; Hogan et al., 1989; Miltenburg et al., 1996; Schukken et al., 1989), as well as their prevalence is known to increase also in humid seasons with relatively high temperature (Sandholm and Pyörälä, 1995a). So this trial was carried out in large-scale dairy herds producing LSCCM for many years, and most of its sampling series were conducted in seasons supposed to provide the most suitable environmental conditions for rapid multiplication of these pathogens in fecal contaminated bedding material. Due to this reason, however, these findings can not be concluded from epidemiological point of view.

The endocrine, metabolic and immune response of high-yielding dairy cows to the postpartum energy imbalance is known to have clear age-related characteristics (Suriyasathaporn et al., 2000). To avoid this source of variance only 2nd parity and older cows were involved in this study. The chosen cut-off value of 1.00 mmol/l BHB in plasma between *hyperketonaemic* (≥1.00 mmol/l) and *normoketonaemic* (<1.00 mmol/l) conditions is in agreement with the literature (Bruss, 1997), and is accepted also by others (Sartorelli et al., 2000) in similar studies. In model trials plasma level of BHB <0.80, 0.8-1.60 and ≥1.60 mmol/l are considered as low, medium and high concentrations, respectively (Suriyasathaporn et al., 1999). The same

border in plasma AcAc is about 0.35 mmol/l (Bruss, 1997; Sartorelli et al., 2000). However, due to its analytical uncertainties the AcAc is considered usually as a less expensive, but also less reliable parameter (Bruss, 1997).

The challenge tests used in this trial for the estimation of functional capacity of adrenocortex and thyroid gland were introduced in the methodology of clinical oriented research in ruminants in the eighties (Alan et al., 1986; Hurley et al., 1981), and have been widely accepted since than (Bage et al., 2000; Kolk, 1991; Romo et al., 1997; Rumsey et al, 1999; Tveit et al., 1990). When standard low doses (60 μg $_{1\text{-}24}$ACTH and 400 μg TRH) are administered for challenge, and ACTH-induced cortisol response and TRH-induced T_4/T_3 response are determined simultaneously, the two methods do not interfere with each other (Kulcsár and Huszenicza, unpublished data).

The pathophysiology of Gram-negative (endotoxin) mastitis

Clinical cases of mastitis caused by GN pathogens (under farm conditions mostly by *E. coli*, but sometimes also by *Klebsiella* ssp., and other *Enterobacteriacae*) may load the udder with endotoxin (lipopolysaccharide, LPS) in severe form. This constituent of the bacterial cell membrane is liberated from killed GN bacteria (Persson et al., 1993; Persson-Waller, 1997). After phagocytes are activated against invading bacteria, they release products, such as complement (C5a), IL-1, IL-6, IL-8 and TNFα. These products are mediators of inflammation, comprising pro-inflammatory cytokines such as IL-1 and TNFα (Persson et al., 1993), and chemo-attractans, such as IL-8 (Barber and Yang, 1998; Boudjellab et al., 1998; Schuster et al., 1997) and C5a (Persson et al., 1993; Schuster et al., 1997; Smits et al., 1998). In response to chemoattractants, PMN adhere to and migrate along the endothelial cell surface towards the site of infection. Migration of blood PMN between endothelial cells into the site of infection is dependent on the number of blood PMN and its migration capacity (Van Werven, 1999). Low number of blood PMN pre-challenge are also related to increased severity of experimental *E. coli* mastitis (Kremer et al., 1993b; Van Werven, 1999; Van Werven et al., 1997). Without an exposure to the chemoattractants, the interaction of adhesive molecules between leukocytes and endothelial surface mediated by E- and P- selectin is weak, but the strong interaction appears after exposure to the chemoattractants. Then integrins on leukocytes, like LFA-1 (CD11a:CD18) and Mac-1 (CD11b:CD18), act on ICAM-1. In consequence, the leukocytes attach firmly to the endothelium and rolling is arrested. In the last step, leukocytes extravasate, and then migrate through the tissue under the influence of the chemoattractant. This step is related to the expression of CD31 in leukocytes and endothelial cells. However, preinfection expression of CD11a and CD11b was not related to the severity of mastitis (Dosogne et al., 1997; Van Werven et al., 1997). The absence of this relation may be due to the increased expression of integrins after infection (Smits et al., 1998). The lack of appearance of chemotactic and CD18- upregulating activities until 12 h after challenge indicated that delays in neutrophil recruitment result from an initial lack of bacterial recognition and inflammatory mediator production (Schuster et al., 1997). Therefore, the upregulation of integrin expression after infection is more closely related to the capacity of udder defense (Schuster et al., 1996). Some substances, for example cortisol, induce a decrease in the expression of CD18 receptors after experimental IMI, modulating the acute inflammatory response in mammary glands of lactating cows (Roets et al., 1999).

Although the usual quantity of endotoxin absorbed form the udder to the blood stream is limited (if any), the above detailed inflammatory mediators and mainly the TNFα are known to be responsible for all the systemic consequences of this disease (Klassing, 1988). These systemic effects are thought to be the most obvious in the *colostral phase* (when the LPS-neutralizing capacity of the udder epithelium is diminished) and / or in *cows suffering from hepatic lipidosis* (when the endotoxin detoxification of the liver is impaired) (Andersen et al., 1996; Sandholm and Pyörälä, 1995a). Within the framework of the current study (1) in cases

caused by *GN environmental pathogens* (*E. coli*, and the sporadic forms of *Klebsiella* ssp., other *Enterobacteriacae*, and *Pseudomonas aeruginosa*), (2) in *NDP mastitis*, as well as (3) in those resulted from *GP contagious* (*S. aureus*) or *environmental* pathogens (*Str. uberis, Str. dysgalactiae and fecal Streptococci*) the *mastitis related endotoxin loading* was considered to be confirmed, supposed or excluded, respectively.

In all of our experiments the endocrine and metabolic alterations seen in cows with GN mastitis were close to our corresponding findings in mastitic cases with no detectable pathogens (NDP). In accordance with the literature (Sandholm and Pyörälä, 1995a) we think that at the beginning the overhelming part of these inflammatory processes was induced by intracysternal GN (mainly *E. coli*) infections, but the original pathogens had been eliminated by the self-defense mechanisms of the udder before sampling. So due to this supposed endotoxin-derived character of this process we pooled the data of cows with GN and NDP mastitis for the final evaluation.

4.2.4.2. Interrelations between energy metabolism and mastitis

Metabolic predisposition for mastitis caused by various pathogens

In epidemiological studies hepatic lipidosis and ketosis were associated with an increased risk of clinical mastitis and other coinciding complications (e.g. endometritis) of bacterial origin (Correa et al., 1993; Erb and Gröhn, 1998; Markusfeld, 1985, Oltanecu and Ekesbo, 1994; Schukken et al., 1988; Valde et al., 1997). Also the course of an experimentally induced *E. coli* mastitis was proved to be more severe in ketotic than in non-ketotic individuals (Kremer et al., 1993a). The effect of NEB and its consequences (hepatic lipidosis, hyperketonaemia) on incidence and course of mastitis can be explained by the impairments caused in antimicrobial self-defense mechanisms. The same decrease in host immunocompetence may predispose the cow also for bacterial complications in uterine involution. In cows with bacterial complications in the postpartum period some defects in PMN functions were noticable even before parturition: Cai et al (1994) observed a prepartum decrease in superoxyde production activity by neutrophils from cows with metritis and in chemotaxis by neutrophils from cows with mastitis. These findings were considered to indicate possible prepartum initiation of the functional defects and impaired neutrophil function as a possible predisposing factor.

The nature of these hepatic lipidosis and hyperketonaemia related impairments are known from several model trials. In vitro studies proved that *diminished phagocytic capacity* of milk PMN granulocytes and macrophages incubated in acetone or BHB containing culture media (Klucinski et al., 1988a). BHB (but not AcAc) at concentrations seen in mild ketosis appeared to inhibit superoxyde anion release from ovine PMNs when submitted to specific and non-specific co-stimulation (Sartorelli et al., 2000). Bovine leukocytes were reported to show suppressed *respiratory burst* activity when cultured in the presence of BHB (Hoeben et al., 1997). In cows with severe hepatic lipidosis (liver triacyl glycerol content: ≥40 mg/g) the antibody-independent and -dependent cellular cytotoxicity of blood PMN was markedly reduced (Zerbe et al., 2000). The function of blood and milk lymphocytes in *response to mitogens* was also impaired in ketotic cows (Kandefer-Szerszen et al., 1992; Klucinski et al., 1988b; Sato et al., 1995; Targowski and Klucinski, 1983). Lymphocytes isolated from ketotic cows were found to produce lower amount of *cytokines*, such as interferons (Filar et al., 1992; Kandefer-Szerszen et al., 1992). So it was hypothesized that the generation of chemoattractants might be reduced in hyperketonaemic cows. BHB and AcAc added in culture media induced *inhibitory effects on the proliferation of bovine bone marrow cells* (Hoeben et al., 1999), and were reported to reduce bovine T-lymphocyte blastogenesis (Franklin et al., 1991). In vitro concentrations of ketone bodies, similar to those observed in mild or severe ketosis were reported to decrease chemotaxis and latex particle uptake in ovine PMN granulocytes (Sartorelli et al., 1999). The magnitude of leukocyte influx was dependent on the *chemotactic*

capacity of leukocytes. In vitro chemotactic differential and chemotactic index of blood PMN were higher in induced ketotic cows (Kremer et al., 1993a). Leucocytes from naturally occurring ketotic cows had lower chemotactic differential than those from non-ketotic cows, and a chemotactic capacity indicated by a chemotactic differential was impaired when leucocytes migrated in an environment with ketone bodies in vitro (Suriyasathaporn et al, 1999). Independently of chemotaxis, *random migration of leukocytes* from non-ketotic cows in cultures containing ketone bodies was significantly decreased, and this effect was also present a bit weaker, in leukocytes from ketotic cows (Suriyasathaporn et al., 1998). Random movement and production of superoxide anions by leukocytes require large amounts of *energy*, the adverse effects of energy metabolism may be related to the impairment of leukocytes function in NEB cows. In addition AcAc and BHB at pathological concentrations in the culture may also interfere with the utilization of glucose for energy. In mouse models, AcAc or BHBA added to culture media were not utilized, as sources of energy by mouse macrophages (Newsholme et al., 1986, 1987). All these factors may be constituents of the temporary immunosuppressive status of ketotic animals.

In *Exp. 2* we wished to collect data on predictive values of certain plasma metabolites and metabolic hormones. For this purpose samples were taken on day 1-3 after calving and several parameters informing us on the current stage of NEB and/or liver function were determined. The cows affected by mastitis as a single disease or in combination with APE some days later in the early or late puerperium showed more elevated AcAc, BHB, NEFA and rT_3, and lower IGF-I, T_4 and T_3 levels then those remained healthy during the first 4 weeks after calving. This tendency related to a more severe form of energy imbalance (Blum et al., 2000; Haraszti et al., 1982; Huszenicza et al., 1988; Kunz and Blum, 1985; Ronge et al., 1988;), and derived mainly from parameters of mastitic cows infected with GP and GN environmental pathogens or affected by NDP mastitis. However, just after calving data of those with *S. aureus* IMI were very close to their healthy herdmates. Calculating the odds ratio we could attribute a significant predictive value only to the elevation of BHB, but not to any others of NEB related changes in circulating levels of hormones and metabolites. This predictive value was highly significant for GN microbes, a bit less obvious for GP environmental pathogens and questionable, if any for contagious pathogens. Based on these findings we suppose that in the early weeks of lactation hyperketonaemia, rather than NEB by itself can predispose cows for mastitis. The pathogen dependent character of this predisposition may be explained by differences in the mechanisms, through which the udder can prevent and/or eliminate various forms of microbial infections. We hypothesize that hyperketonaemia may depress mainly those components of the antimicrobial self-defense, which are responsible for destruction of invading environmental pathogens entered passively into the cisternal system and/or into the lactiferous ducts. *S. aureus*, however, is able to colonize actively the teat apex, first of all in cases of epithelial injury. After adhesion these bacteria can adapt to the milk environment forming a mucopolysaccharide capsule to avoid phagocytosis. After penetration through the teat barrier into the cistern the invading pathogen is able to adhere to milk fat globules and can float upwards in the udder (Pyörälä, 1995a). In accordance with the literature (Pyörälä, 1995a; Zadoks et al., 2001) we think that several other factors [e.g. some kinds of lowered resistance: changes in environmental temperature, virus infections (bovine herpesvirus 4 and others), recovery from a preceding mastitis, other infected quarter(s) of the same cow, extremely callused teat ends, and epithelial erosions] rather than the hyperketonaemia related impairments in leukocyte function seem to be the primary factors predisposing the cow for *S. aureus* mastitis. The machine milking associated teat-end condition (callosity) may be also an important constituent of the predisposition also in case of environmental pathogens (Neijenhuis et al., 2001). However, in the later group a protective effect is attributed to the presence of monocytes and PMN granulocytes in the milk of the teat cistern, and also their functional capacity play a significant role in rapid elimination of invading pathogens, and so in diminishing the

clinical consequences of IMI (Beaudeau et al., 2002). These components of the self-defense mechanisms may be affected by hyperketonaemia in the early days of lactation.

Metabolic changes after the mastitis outbreak

In accordance with the above findings, in *Exp. 3*. we could also detect elevated BHB levels in the first samples of cows with NDP+GN mastitis taken within some hours after the outbreak of clinical symptoms. The BHB dependent character of NDP+GN mastitis was obvious in the first four weeks after calving, but seemed to disappear later in the peak lactation. During the sampling period BHB levels started to decrease and reached the physiological range within hours. After intravenous administration of 2 μg/kg body weight LPS a similar reduction of circulating BHB level was also seen in non-lactating heifers (Steiger et al., 1999), which was thought to reflect an inhibition of hepatic ketogenesis rather than a reduction of ruminal ketone production due to reduced feed intake (Huhtanen et al., 1993). Under the conditions of the current study the ketone reducing effect of these factors might be completed also by the metabolic consequences of the mastitis-induced sharp drop in milk production (not studied in this trial). Neither the present observations, nor the data of earlier model studies (Steiger et al., 1999) reveal whether an endotoxin-induced stimulation of ketone body utilization (Memon et al, 1992) also contributes to the decrease in plasma BHB.

Contrary to this continuously decreasing tendency in BHB, a temporary elevation of NEFA level was seen in cows with NDP+GN mastitis in the first some hours of the course, but not in those with GP mastitis. In cattle model studies a similar LPS- or TNFα-induced some-hour-long elevation was observed in the plasma levels of free fatty acids, which was completed coincidingly with a momentary reduction in triglyceride concentration, a biphasic growth of plasma glycerol, a dramatic increase in plasma lactate content, as well as with a temporary elevation followed by a significant reduction in blood glucose level (Kushibiki et al., 2000, 2001a and 2001b; Steiger et al., 1999). Endotoxin-induced severe disruptions in glucose homeostasis have been verified by several other studies in lab rodent and human models (Berczi, 1993), as well as in ruminants (Elsasser et al., 1996; Giri et al., 1990). In accordance with others (Elsasser et al., 1995, Sartin et al., 1998, Steiger et al., 1999) we think, that the NEFA increase observed also in the current study (*Exp. 3*) might be the catabolic consequence of endotoxin induced endocrine changes (mainly of the temporary increase in serum levels of catecholamines and/or in degree of insulin resistance).

4.2.4.3. The adrenocortical function of postpartum dairy cows and its interference with various forms of mastitis

Glucocorticoid production

Our knowledge on the macroscopic lesions of the bovine adrenal cortex is based mostly on abattoir studies. Hamir and Parry (1980) in Canada reported that under that conditions the different (malignant, benign) adrenocortical neoplasias take the second place behind palpebral carcinoma in the frequency order of bovine tumorous diseases: every 6.7 cases of 100 tumorous diseases diagnosed in the slaughter houses, were localized in the adrenal cortex.

It can be stated that the malfunctions of adrenal cortex in cattle with no obvious macroscopic alterations usually remaining hidden in abattoir examinations occur considerably more frequently than the adrenocortical neoplasia. Above all, the provisional decrease in cortisol responsiveness to a standard low-dose ACTH challenge (*temporary hypocorticism*) reported to occur around parturition and during the first few weeks after calving may be worth mentioning (Kolk, 1991). Although there have been several observations about its occurrence, we hardly know anything yet about its real prevalence rate, the (nutritional and other) factors affecting its incidence and duration, and its pathological significance. Also, it is practically unknown whether there is any relationship with the malfunctions of the thyroid gland and/or

pancreas (the impaired function of these latter two endocrine glands is also supposed to be relatively frequent phenomenon in this period) (Holtenius and Traven, 1990; Holtenius et al., 1993, 1996; Hove, 1978; Kapp et al., 1979; Pethes et al., 1985).

Simultaneously with some surgical interventions and obstetrical diseases (acute mastitis and metritis, retained placenta, prolapsed uterus, milk fever) higher glucocorticoid levels can be measured in the blood (Waage, 1984). This postpartum stress and the subsequent peak of cortisol level have a negative effect on pituitary responsiveness to GnRH stimulation during the first week (Torres et al, 1997a). In a study on postpartum cows, Torres et al. (1997b) found an association between elevated basal plasma cortisol concentration and abnormal puerperium. These abnormal cows showed a higher rate in delayed uterine involution. Although the cortisol concentration is not influenced by subclinical mastitis (Paape et al., 1974). In some cases this rise in blood cortisol level is probably caused by nothing else but the endogenous stress coinciding with these conditions (prolapsed uterus or milk fever). In other cases (acute mastitis accompanied by serious general symptoms, and perhaps also the acute putrid endometritis), however, it may be supposed that the endotoxin and some mediators of the inflammatory reaction absorbed from the site of lesion (udder, uterus) are responsible for the increase in the cortisol level. Among these mediators certain interleukins and the TNFα are known to play a central role, which – when administered in appropriate doses – can, in itself, create nearly all of the symptoms of the so-called *Gram negative toxic shock syndrome* (Klassing, 1988). Cortisol and its potent analogs inhibit the transcription of IL-1β (Lee et al., 1988), and can block increased concentrations of TNFα and reduce concentrations of solubile TNFα receptors and IL-1 receptor antagonist in a time-dependent manner (Barber et al., 1993, 1995) in rodent and human models. So the LPS-induced cortisol increase can be considered as an important physiological self-defense reaction reminding to a specific 'feedback-like' mechanism in the down-regulation of TNFα (over)production, by means of which the organism tries to avoid the excessive – destructive to the organism itself – formation of this substance. It has been recently observed (Roets et al., 1999) that cortisol can induce a decrease in the expression of CD18 receptors after experimental IMI, modulating the acute inflammatory response in mammary glands of lactating cows. Reduced glucocorticoid receptor expression in blood neutrophils of periparturient dairy cows was associated with increased serum cortisol concentrations, leucocytosis, and neutrophilia. Thus, glucocorticoid receptor downregulation in neutrophils may be involved in periparturient neutrophil dysregulation and may cause increased susceptibility to mastitis (Preisler et al., 2000). It is well-known that the general toxic effects of bacterial LPS depend on the amount entering the circulation and the clearance capacity of the organism (Bertók 1998). However, LPS is generally not absorbed from the mastitic udder into the blood circulation, the reaction being induced by cytokines such as IL-1 and -6 and TNFα (Jackson et al. 1990; Sandholm and Pyoräla 1995a). Experimental LPS administration induces TNFα production and glucocorticoid release within 1 h and 3 h respectively (Elsasser et al. 1995; McMahon et al., 1998, Steiger et al., 1999). In one of our earlier model studies we could confirm this phenomenon also after intramammary administration of endotoxin (Hirvonen et al., 1999). In cattle with normal adrenocortical responsiveness, the increase in plasma cortisol level starts already a few minutes after the beginning of the endoxin effect, it reaches a *peak concentration after 3-4 hours and does not last longer (except for fatal cases) than 24 hours*. During this time, however, the plasma level of endotoxin stimulated acute phase reactants including the TNFα dropped to basal level (Dickson, 1990; Hardie and Kruse-Elliot, 1990; Hirvonen et al., 1999; McMahon et al., 1998; Sandholm., 1995b; Schuster and Harmon, 1992; Sordillo and Peel, 1992; Steiger et al., 1999). Endoxin-induced increase in the cortisol level can be achieved by the activation of the hypothalamus-anterior pituitary axis [in a corticotrop releasing hormone (CRH)- and ACTH-dependent way] and also independently of this system (Elenkov et al., 1992; Rivier and Rivest, 1991;). The latter possibility was also proved in ruminants not a long time ago (Bosu et al., 1995): it has become known recently that the steroid production of adrenal cortex can also increase independently

of the CRH+ACTH system, through the direct mediation of bacterial endotoxin and/or the subsequent release of these mediator substances. Thus temporary increase can be observed not only in the plasma level of cortisol, but, in severe cases, also that of other steroids (e.g. progesterone).

Among others the *temporary hypocorticism* may supposedly have pathological importance in the development, severity and/or course of certain endoxin-mediated diseases (including the *E. coli* / coliform mastitis and perhaps also the APE) occurring in the early weeks of lactation. However, up to now the question of its real involvement in the pathogenesis of these diseases has remained still unanswered. About 23 % of the cows in *Exp. 2* showed < 40.00 nmol/l (e.g. lower than the mean - SD of symptomless, normoketonaemic cows; n=199) cortisol response to the ACTH challenge. After Kolk (1991) this low response was considered as a sign confirming *temporary hypocorticism*. At the time of challenge all of the cows were healthy, and neither the baseline level of cortisol, nor the degree of ACTH-induced cortisol response predisposed them for mastitis. However, if *hypocorticoid* cows were affected by GN or NDP mastitis in the first 14 days after calving, they showed more severe clinical symptoms and had higher risk for a fatal course, than their normocorticoid counterparts. These observations are in complete agreement with earlier experiences obtained in lab rodents and in the human medicine (Berczi, 1993): Adrenalectomized mice showed an extreme sensitivity to the lethal effect of experimental LPS challenge, and responded with an exaggerated TNFα production. High rate of human patients with severe GN septicaemia and not responding adequately to standard therapy were also reported to have adrenocortical insufficiency.

In accordance with one of our earlier findings in a model study (Hirvonen et al., 1999) we could also detect the LPS-induced cortisol increase in *Exp. 1, 3* and *4* of the current trial. In *Exp. 1* all six cows with identified GN mastitis showed elevated cortisol concentrations (>10 nmol/l; results not shown) at some time during the infection, whereas values were below this level at all times in the five healthy cows and those with mastitis caused by GP pathogens. Increased cortisol concentrations were associated with low T_4 concentrations independently of the relationship between T_4 and T_3 or IGF-I. At the beginning of the sampling process elevated levels of cortisol were seen in cows with NDP+GN mastitis in *Exp. 3* and *4*, as well. According to the design of these studies the first samples taken at 14.00 h represented the first about 7-14 h of the clinical course. In *Exp. 3* less increased, but still high group mean of cortisol concentrations were detected 6 h later (e.g. at the 13-20th h of the course). However, the standard deviation was high. Then the plasma level of cortisol returned to the physiological range. In complete agreement with these experiences in model studies the cortisol in plasma started to increase a few minutes after the endotoxin challenge, it reached its peak concentration after 3-4 h, and its duration did not exceed 24 h (Dickson, 1990; Hardie and Kruse-Elliot, 1990; Hirvonen et al., 1999; McMahon et al., 1998; Schuster and Harmon, 1992; Sordillo and Peel, 1992; Steiger et al., 1999). However, the fatal cases showed the most pronounced, about ≥24 h-long cortisol increase in these cited model studies, but not in our cases: in the 3 cows died in *Exp. 3*. no cortisol increase was detected at all. Comparing the experimental animals low-yielding late-lactation individuals were involved in the various model studies cited, but all 3 cases died in *Exp. 3* were in the earliest few days after calving. In accordance with findings of Kolk (1991) our cows in *Exp. 4* showed significantly lower ACTH-induced cortisol response in the early puerperium, than in the later stages of lactation. The two cows died of *E. coli* and NDP mastitis could hardly response to the ACTH challenge. The clinical outbreak of mastitis was diagnosed in both of them also in the early puerperium. In cows with NDP+GN mastitis in the early puerperal phase the ACTH challenged cortisol increment inversely related to the severity of clinical symptoms.

We think, these experiences confirmed the regulatory role of physiological cortisol response in production and release of certain interleukins and TNFα in GN mastitis, emphasizing the clinical importance of temporary hypocorticism in postpartum dairy cows. So beside

the glucocorticoid receptor down-regulation in neutrophil granulocytes (Preisler et al., 2000), it may be the other alternative how the glucocorticoids may relate to pathogenesis of mastitis.

Progesterone, as a side product of forced cortisol synthesis

In *Exp. 3*, simultaneously with the LPS-induced cortisol elevation also a slight, temporary *progesterone* (P_4) increase was seen in cows with NDP+GN mastitis, maintaining the supra-physiological plasma concentration of this hormone for some hours only. This temporary P_4 increment was detectable only when no active corpus luteum was present on the ovary, and it was considered to be of adrenal origin as a side product of the LPS-forced cortisol synthesis. A similar temporary P_4 increase was reported to occur also in ovariectomized heifers after a standard low-dose ACTH challenge (Bage et al. 2000), and this finding was concluded that a sustained adrenal stimulation associated with environmental or social stress could be one of the factors in repeat breeding syndrome. Our observation in mastitic cows completes the list of these factors with the endotoxin (GN+NDP) mastitis.

4.2.4.4. The thyroid function of postpartum dairy cows and its mastitis related alterations

Thyroid hormones are important regulators of homeostasis including the energy and protein metabolism. T_4, the predominant product of the thyroid gland may undergo extrathyroidal enzymatic activation (e.g. outer-ring deiodination) by 5'-deiodinase (5'D) producing T_3, or inactivation (e.g. inner-ring deiodination) by 5-deiodinase (5D) producing rT_3. All three thyroid hormones are present in the circulation, however inherent physiological effects are attributed only to T_3 (Dickson, 1990; Leonard and Visser, 1986). The positive correlation between circulating thyroid hormone concentrations and energy balance is well-known in many species including cattle (Capuco et al., 2001; Cassar-Malek et al., 2001; Janan et al., 1995; Kunz and Blum 1985; Pethes et al. 1985; Ronge et al., 1988). It is probable that alterations in plasma levels of T_4 primarily reflect changes in thyroid secretion rate (Riis and Madsen 1985), as well as the balance of T_4 activation and inactivation associated with the energy metabolism (Pethes et al. 1985; Capuco et al., 2001; Cassar-Malek et al., 2001). Peripheral T_3 concentrations are influenced mainly by extrathyroidal 5'D activity. Because T_3 is a potent regulator of energy and protein metabolism, the extrathyoidal activity of 5'D (and perhaps also of 5D) is an important control point for regulating the metabolic status (Kaplan, 1986). In cow, the highly efficient type II 5'D dominates in the mammary gland enabling T_3 production in support of lactation to proceed at the expense of other tissues, such as the liver, where type I 5'D prevails (Ślebodziński et al. 1999). In cattle plasma levels of thyroid hormones may be altered also by other nutrition- and metabolism-related factors, such as selenium and/or iodine deficiency / supplementation (Awadeh et al., 1998; Wichtel et al., 1996), growth hormone releasing factor and somatotropin administration (Kahl et al., 1995), providing fat- or starch-enriched diet (Blum et al., 2000; Bunting et al., 1996; Romo et al., 1997), and feed contaminants, for instance goitrogens (Bernal et al., 1999; Gennano-Soffietti et al., 1988; Thrift et al., 1999) and certain ergot like alkaloids produced by endophyte fungi (*Neotyphodium coenophialum*) of tall fescue (*Festuca arundinacea*; known as an existing form on feed contamination only in North America) (Browning et al., 1998 and 2000; Hurley et al, 1981).

In accordance with the nutritional and metabolic influences in late-pregnant, dry cows relatively high concentrations of thyroid hormones were detected, followed by a significant decrease in the periparturient period. Blood levels of T_4 were found to be lower in the earliest days of lactation than in late lactation (Kesler et al., 1981; Pethes et al., 1985; Tiiras, 1997). Plasma concentrations of both T_3 and rT_3 were in their nadir in these day, possibly owing to increased metabolic clearance of thyroid hormones in peripheral tissues and/or to suppressed secretory capacity of the thyroid gland. Supporting this idea the TRH-induced T_4 and T_3 re-

sponses were also less pronounced in the 2nd week of lactation than before calving, or 3 months postpartum (Tveit et al., 1990).

TNFα is reported to decrease 5'D activity in peripheral tissues during starvation, as well as in low T_3 syndrome (see below) (Pang et al., 1989), and may play a regulatory role in many other physiological states (Bartalena et al., 1998). A number of infectious and inflammatory diseases are associated with profound changes in thyroid status in mammals including domestic animals (Lohuis et al., 1988). This so-called *euthyroid sick syndrome* (syn.: *low T_3 syndrome*) is observed during systemic non-thyroidal illnesses, and consists of a decrease in plasma concentration of T_3, an increase in rT_3 level and, in severe cases, a decrease in T_4 and thyrotropin (TSH) concentrations. Most of these changes are caused by a lower T_3 production rate and a decreased rT_3 clearance rate due to the diminished extrathyroidal 5'D activity (Wartofsky and Burman, 1982). During the inflammatory process some of the citokynes (TNFα, IL-1) are important mediators of changes in thyroid status (e.g. inhibition of TSH release from pituitary cells and decreased activity of type-I 5'D in thyroid and liver tissue) (Haastaren et al., 1994; Hashimoto et al., 1995; Pang et al., 1989). Endotoxin exposition is a strong stimulus for cytokine release, reducing the production and circulating level of T_4 and inhibiting the T_4 deiodination to T_3 in many species, including lab rodents, humans and also ruminants (Bartalena et al., 1998; Berczi, 1993; Bertók 1998; Kahl et al. 2000; Lohuis et al., 1988; Nagy et al., 1983). As reviewed by Bartalena et al. (1998) almost all steps of thyroid hormone synthesis, secretion and peripheral metabolism may be negatively influenced by this endotoxin-induced cytokine release. In rats the shock-inducing dose of endotoxin inhibited the TSH-challenged T_4 response, due to the membrane damaging effect of LPS (Nagy et al., 1983). However, up to now similar studies have not been carried out yet in ruminants.

In *Exp. 3* and *4* the thyroid gland function and T_4/T_3 metabolism of our high-yielding postpartum dairy cows were subjected to mastitis-induced natural release of cytokines. This cytokine loading might have been the most intensive in the severe forms of NDP+GN mastitis, and as in the model study of Kahl et al. (2000), it was capable of inducing a significant decrease in plasma levels of both the T_4 and T_3 (in *Exp. 3*), and diminished also the TRH-challenged T_4 and T_3 increase (in *Exp. 4*). These mastitis related changes were more obvious in more advanced stages of the course (in *Exp. 3*), were more pronounced in the early puerperium than in the late puerperium or during the peak lactation (both in *Exp. 3* and *4*), and were extremely dramatic in the few cases died of mastitis soon after sampling. This latter observation is comparable to the earlier findings in rats treated with shock-inducing dose of endotoxin (Nagy et al., 1983). Contrary to the experiences in cows with NDP+GN mastitis, only mild or no mastitis-induced alterations were detected in animals affected by GP mastitis.

In *Exp. 4* also the rT_3 concentrations were determined in the pre-challenge (t_0) samples taken in the first about 7-14 h of the clinical course. The rT_3 levels of healthy animals were in about the same range as in the literature (Pethes et al., 1985; Tiiras, 1997), but contrary to these finding in our study the cows sampled in the early puerperium showed more elevated concentrations than those sampled later. This discrepancy might be explained with the differences in milk production: In their previous lactation the cows of these cited studies produced about 5000-5500 kg milk in average, however, all of our experimental animals yielded more than 6500 kg (the mean varied between 7500 and 8100 in the different experiments). Comparing to the corresponding data of healthy ones, as well as of those with GP mastitis, the cows with NDP+GN mastitis were characterized by significantly more elevated rT_3 levels. These – supposedly endotoxin mediated – differences derived from the data of the most severe cases. This observation reveals that due to a mastitis related endotoxin loading in cows not only the 5'D-dependent activation of T_4 to T_3 may be impaired (Kahl et al. 2000), but also the capacity of its 5D-catalyzed inactivating pathway to rT3 can be increased: this may be a significant contribution for the LPS-induced decrease of T_4 in plasma.

In *Exp. 1* after a short peri-parturient depression the mean T_4 concentration had returned to the initial value within 5 days postpartum in the group of cows without mastitis but not in

the *E. coli* infected group. Very low thyroid hormone concentration was detected in three cows (T_3 – 0.35 nmol/l and T_4 – 8.5, 10.9 and 12.5 nmol/l) already before calving, which was followed by the mastitis outbreak only some hours or days after delivery. The low prepartum T_4 levels in the cows which later exhibited supposed or proved forms of GN mastitis may indicate either that cows with low T_4 status were more susceptible to infection by GN organisms or that the endotoxin-released products were already acting on the thyroid gland before clinical mastitis was evident. This latter version seems to be quite improbable in the light of findings in *Exp. 2*, where we could detect low T_4 and T_3 levels on day 1 to 3 after calving in cows affected by mastitis some days or 1 to 4 weeks later only. In the first few days of the lactation these low thyroid hormone levels may associate mainly with the presence and degree of NEB (Capuco et al., 2001; Cassar-Malek et al., 2001; Janan et al., 1995; Kunz and Blum 1985; Pethes et al. 1985; Ronge et al., 1988). We think, the immunosuppressive consequences of decompensated NEB, rather than the low plasma levels of T_4 and T_3 by themselves predisposed our cows for mastitis in *Exp. 1* and *2*.

4.2.4.5. Functional characteristics of growth hormone (GH), insulin-like growth factors (IGFs) and insulin in dairy cows and their mastitis related alterations

GH and IGFs are known as generally anabolic hormones that act to regulate whole body growth, metabolic tissue responses and cellular survival. They have also been implicated in the regulation and functioning of the immune system. GH has direct effects on cell growth and differentiation, but many of its growth-promoting actions are also via the stimulation of IGF-I synthesis and secretion, notably from the liver. GH acts to stimulate productive processes such as growth and milk synthesis and promote homeorhetic adaptations to provide nutrients for these processes (Dickson, 1990; Mol and Rijnberk, 1997).

IGF-I (also known as somatomedin-C) was originally thought to be only an exclusive mediator or second messenger of GH (Salmon & Daughaday, 1957). GH is undoubtedly the prime regulator of IGF-I production by the liver but nutrition also has a substantial influence on production and plasma level of IGF-I. IGF-I is produced in most tissues and acts in an autocrine, paracrine or endocrine manner through the type 1 IGF receptor (IGF-IR), or to a lesser extent, the insulin receptor. IGF-I is secreted as it is produced and the highest concentrations are found in the blood (Thissen et al*.,* 1994). The actions of IGF-I in regulating postnatal growth and protein, carbohydrate and bone metabolism are either enhanced or inhibited by at least 6 different soluble high-affinity binding proteins (IGFBPs 1-6) circulating in the blood (Rajaram et al., 1997; Russell-Jones and Umpleby, 1996; Skaar et al. 1991; Vega et al. 1991).

Insulin and IGF-I have similar metabolic effects: they cause cellular hypertrophy (increase in size) but despite this functional overlap there are different consequences to their action (McCusker, 1998). Insulin-induced hypertrophy acts as a means to increase nutrient stores and occurs relatively acutely. The prolonged effect of IGF is important for cell survival, hyperplasia (increase in number) and subsequent differentiation.

However, metabolic responses (inducible by insulin or IGF-I) need to be curtailed when nutrients are limited or environmental stressors are acting on the organism. Insulin-induced effects are acute and reversible, an adaptation presumably evolved due to episodic feeding and hence more likely to be important for the rapid regulation of metabolism (Ronge et al., 1988). Insulin increases rapidly postprandially in response to elevated glucose concentrations but is quickly removed from the circulation as nutrient uptake occurs rapidly. Insulin concentrations are typically within the picomolar range and the hormone demonstrates no binding affinity with IGFBPs thus freely circulates. IGF-I concentrations do not fluctuate with feeding activity or diurnally (Ronge et al., 1988). Actions resulting from IGF-I stimulation, i.e. mitosis or differentiation, require anything from 6 hours to several days to complete (McCusker, 1998). Thus IGF-I concentrations must be maintained for such events to be completed. This is

partly achieved by the IGFBPs that help to maintain a pool of IGF in the circulation (Hossner et al., 1997). IGF concentrations tend to be higher than those of insulin, within the nanomolar range and the ligands are "unavailable" for interaction with their receptors whilst bound to IGFBPs.
The regulation and function of IGFBPs has recently been reviewed by Rajaram et al. (1997). IGFBP-1 is the only IGFBP known to be subject to diurnal variation, it is nutrient sensitive and concentrations in blood can change rapidly (increase with fasting but decrease to basal within 2 hours of re-feeding (Rajaram et al. 1997).

In dairy cows the serum levels of insulin and IGF-1 were reported to relate inversely to energy and protein supply (Ronge et al., 1988). The levels of energy were more important than protein in influencing the *postpartum* concentrations of IGF-I and insulin in this study. However, the energy and protein balances of the cows are strongly correlated, making it difficult to separate their individual effects. It may be that interactions in the rumen make it more difficult to demonstrate specific effects of energy or protein on IGF-I concentrations compared with non-ruminant animals (McGuire et al., 1992 and 1995a). Low protein diets in cows were associated with reduced IGF-I by VandeHaar et al. (1999). The source of the protein is important as essential amino acids had a positive effect on IGF-I concentrations and nitrogen balance (T'hissen et al., 1994). Circulating insulin concentrations are a stimulus for IGF-I production (McGuire et al., 1995b) but are reduced when dietary protein is restricted (Fliesen, 1989). When conditions are optimal for IGF-I production, increases are restrained to a greater degree than are decreases induced by suboptimal conditions (Forbes et al., 1989). The addition of bovine GH may have beneficial effects in increasing IGF-I concentrations; however it is likely that it will have detrimental effects on an animal's ability to gain body-weight and body condition score, especially in lactation.

In the light of the above studies the insulin and IGF-I levels of our cows in *Exp. 2* reflected the energy plus protein (but mainly the energy) balance, and the low day 1-3 level of IGF-I in cows showing mastitis some days later confirm only the higher susceptibility of these individuals to intramammary infection caused by environmental pathogens in the early weeks of lactation.

On the other hand the GH/IGF axis was reported to interrelate closely with also the thyroid function (Hoshino et al. 1991, Svanberg et al. 2001). Thus, thyroid hormone status may influence feed intake, which subsequently affects IGF-I levels, and may modify IGF-I concentrations through effects on GH secretion or receptor levels. On the other hand, administration of GH to lactating cows increased the activity of mammary 5'D twofold (Capuco et al. 1989), thus enhancing the metabolic priority of the udder (Kahl et al. 1995). Significant independent associations of serum T_4 with T_3 and IGF-I levels during the puerperium were found in two experiments involving 60 healthy cows (Nikolić et al. 2001b). This relationship occurred again in *Exp. 1* and *2* of the present study. However, IGF-II concentrations emerged as the significant second factor postpartum. This implies that bacterial infection may lead to alterations in the predominant pathways of interaction between different hormonal axes.

GH potentiates the biological activity of endotoxin in the rat (Liao et al., 1997); bacterial endotoxin rapidly reduces blood IGF-I concentrations, which may affect gut barrier function, allowing increased bacterial translocation. In lab rodent and human models insulin is usually elevated in endotoxaemia and GN infection and may be either elevated or decreased during the acute phase response. Glucagon is always elevated, usually in proportion to the severity of condition. Insulin has an influence on the production of cytokines such as IL-6 and TNFα, and in turn cytokines (IL-1, TNFα) regulate the pancreatic insulin production and release. Septic patients did not respond to standard dose treatment with human GH by elevated serum levels of IGF-I, whereas normal individuals did. However, the role of these hormones is supposed to be more important in the metabolic response to infection than in the direct regulation of cytokines (Berczi, 1993).

It is widely accepted nowadays that the endotoxin-induced changes of both the GH-IGF-I axis and insulin participate in the shift of the metabolism towards catabolic events also in ruminants including the postpartum dairy cows. In young and growing calves, in heifers and steers, as well as in a sheep model the experimental intravenous administration of LPS was followed by a significant but only transient increase in insulin level (coincidingly with the cortisol and TNFα elevation), a less obvious growth (in 1-week old calves: a slight reduction) in the circulating GH concentration, and a slower, but marked and relatively long-lasting decrease in IGF-I and IGFBP-2 concentrations (Briard et al, 2000; Elsasser et al., 1995; Kenison et al, 1991; Kinsbergen et al, 1994; McMahon et al, 1998; Steiger et al., 1999). After a temporary hyperglycaemia decreased glucose levels and insulin resistance were observed 6 and 24 h after the LPS challenge (McMahon et al, 1998). A simultaneous decrease in IGF-I and a moderate elevation in IGFBP-1 plasma levels contrasted with the increase in GH secretion suggest that the endotoxin loading causes also a state of resistance to GH, which is exacerbated by a simultaneous reduction in IGF-I bioaviability (Briard et al, 2000). Similar changes in glucose and insulin levels, as well as in insulin resistance were induced also by intravenous administration of TNFα (Kushibiki et al., 2000, 2001a and 2001b).

This temporary elevation in plasma insulin was usually observed also in the first 2-3 samples of our cases with NDP+GN mastitis in *Exp. 3*. In complete agreement with the findings of model studies the IGF-I level was still almost unaffected at the time of this insulin elevation, and started to decrease continuously thereafter. Similar changes in insulin and IGF-I were seen almost never in cows with GP mastitis.

4.2.5. CONCLUSION. IMPLICATIONS

Taking all these experiences into account, we think that the presented results widened significantly our knowledge on factors influencing the incidence and course of bovine mastitis occurring under herd conditions in early weeks of lactation.

The results of *Exp. 1* verify that the act of parturition in cows is accompanied by marked changes not only in circulating levels of sexual hormones (progesterone, estrogens) and cortisol, but also in some other endocrine factors involved in the homeostatic and/or homeorhetic regulation of energy and protein metabolism. The general trends, such as the continuous and final decline in plasma progesterone concentration, the peak in plasma cortisol and trough in insulin concentrations at the onset of parturition, as well as a significant periparturient reduction in thyroid hormone (T_4 and T_3) and IGF-I levels confirmed earlier observations (Hydbring et al. 1999; Schams et al. 1991; Skaar et al. 1991; Stabenfeldt and Edqvist, 1990; Vega et al. 1991). However, in the current data set there were great differences between the individual, at least partly due to the various (mainly GN) forms of mastitis.

Parameters informing on functional characteristics of monocytes and PMN neutrophil granulocytes were not determined in this study, so the conclusive value of our findings in *Exp. 2*. is limited only. However, according to the clear tendencies in our experiences we think, that the high incidence of mastitis caused by environmental pathogens in early weeks of lactation may be the consequence of the NEB-induced decrease in host's cellular immuncompetence. The ketone body (BHB) elevation in the first few days after calving may be one of the crucial factors of this metabolic susceptibility. The same impairments in antimicrobial self-defense mechanisms may predispose the cows also for coinciding bacterial complications in uterine involution (*acute putrid endometritis*). On the other hand, contrary to our expectation under our conditions no metabolic inclination was confirmed for new cases of mastitis caused by contagious pathogens (*S. aureus* in this study), perhaps due to the differences in pathogenesis of these two main forms of mastitis.

Our observations in *Exp. 2, 3* and *4* could clearly demonstrate the involvement of cortisol, as well as of the functional capacity of adrenocortex in the pathogenesis of GN mastitis. In complete agreement with experiences obtained in septic patients in the human medicine (Ber-

czi, 1993) the grade of endotoxin-induced cortisol response seems to be a critical step in pathogenesis: the peak level of cortisol should reach a threshold for triggering the effective down-regulation of further citokyne (first of all TNFα) production and release. This mechanism is known to be crucial in prevention of TNFα overproduction (and so in avoidance of life-threatening exacerbation; reviewed by Sandholm, 1995b), and based on our current data it appears to be one of the rate-limiting factors influencing the course of GN (endotoxin) mastitis in certain cows in the earliest weeks of lactation. These findings justify the clinical importance of anti-inflammatory therapy in mastitic cows showing severe general symptoms of this disease. (It means usually the systemic administration of non-steroidal anti-inflammatory drugs and antihistamins, but also the systemic use of glucocorticoids in the life-threatening cases). Further studies are needed, however, to identify the reason of lower than normal cortisol responsiveness of certain cows in the immediate postpartum period.

Although the induced peak concentrations of cortisol, insulin and NEFA were not so high, and also the individual variation was quite pronounced in all the hormone and metabolite levels due to the relatively less standardized field conditions (e.g. individual differences in mass of endotoxin released, as well as in the time of clinical outbreak before sampling), during the clinical course we could detect the same tendencies in changes of plasma cortisol, T_4, T_3, IGF-I, insulin, BHB and NEFA levels of cows affected by natural outbreak of NDP and GN mastitis (*Exp. 3*), as seen in model studies after an experimental LPS challenge. The TRH-challenged T_4 and T_3 response was also reduced in NDP and GN mastitis (*Exp. 4*). In both the *Exp. 3* and *4* the most obvious endocrine and metabolic alterations were found in the early puerperal phase, and in cows with the most severe forms of NDP and GN mastitis. These changes were extremely dramatic in cases died of NDP or GN mastitis soon after sampling (although the standard dose TRH challenge was capable of inducing certain T_4 and T_3 increment even in these fatal cases), so we think they certainly have relevance in pathogenesis, as well as in regulation of the course. No similar mastitis related changes were seen, however, in case of GP intramammary infections.

From physiological point of view it is remarkable, that in cows with endotoxin (NDP or GN) mastitis not only the 5'D-dependent activation of T_4 to T_3 may be impaired (as demonstrated by Kahl et al. 2000), but also the capacity of its 5D-catalyzed inactivating pathway to rT_3 can be increased (*Exp. 4*).

Although the final improvement of this phenomenon requires further studies, these endotoxin related endocrine and metabolic alterations are supposed to impair also the ovarian function and reproductive performance [as reported in preliminary form by Huszenicza et al. (1998), as well as based on data of their model studies also by Hockett et al. (2000), Oliver et al. (2000), and Suzuki et al. (2001)]. So it may be justified to exclude the mastitic cows (or at least of those with sever general symptoms) from trials studying the endocrine, metabolic and reproductive effects of certain feeding technologies and/or treatment procedures.

5. CHAPTER 2

DRYING OFF THERAPY IN THE CONTROL OF BOVINE MASTITIS

5.1. THE USE OF THE DRY COW THERAPY IN THE CONTROL OF BOVINE MASTITIS: A REVIEW

5.1.1. DRYING OFF AND THE ROLE OF NONLACTATING PERIOD

The nonlactating "dry" phase of the dairy cow is a specific period between two active lactating phases when the mammary gland changes dinamically both in structure and function. Benefits derived from a dry period involve more than improvements in the cow's nutritional status for the forthcoming lactation. Several studies (Akers and Nickerson, 1983; Nickerson and Akers, 1983; Sordillo et al., 1984a; Sordillo et al., 1984b) have shown that adequate proliferation and differentiation of the mammary secretory epithelium during nonlactating period were essential for optimal synthetic and secretory functions in the ensuing lactation of cows. Coppock et al. (1974) found that dairy cows with 10- to 40-days nonlactating periods produced significantly less milk in the subsequent lactation than cows with a 40- to 60 day nonlactating period. Smith and Todhunter (1982) suggested three distinct stages during the nonlactating period: 1) period of active involution that begins with cessation of milking, 2) period of steady state involution that represents the time when mammary glands are fully involuted, 3) period of colostrum formation and the initiation of lactation. Based upon observed changes in bovine mammary tissue and secretion composition, the process of active involution is most likely completed by 21 days after drying off. This period is associated with an abrupt cessation of milk removal, engorgement of cisternal spaces, ducts, and alveoli with milk constituents, marked changes in mammary secretion composition, and regression of secretory tissue. The duration of steady state involution depends on the length of the nonlactating interval. Smith and Todhunter (1982) indicated that a minimal length of steady state involution may result in a decreased hormonally medicated lactogenic response which could be related to suboptimal production in the following lactation in cows with nonlactating periods of shorter than 40 days. Near parturition, mammary glands again undergo marked transition characterized by rapid differentiation of secretory tissue, intense growth, copious synthesis and secretion of proteins, fat, and carbohydrates, and accumulation of colostrum (Oliver and Sordillo, 1988). The greatest increase in mammary DNA content of heifers occured in the last trimester of pregnancy (Swanson and Poffenbarger, 1979). Morphogenesis of secretory capability in bovine mammary glands also became evident during the last few weeks of gestation (Sordillo and Nickerson, 1988).

A classic study by Neave et al., (1950) demonstrated that mammary glands were markedly susceptible to new intramammary infections (IMI) during the early dry period. After cessation of milking several important changes may affect susceptibility of mammary glands to new IMI: 1) the flushing effect of milking on bacteria colonizing the teat canal is terminated, 2) increased intramammary pressure that may cause leakage of milk and facilitate bacterial penetration of the streak canal, 3) the defence mechanisms of the mammary gland are at low level during early involution: low numbers of polymorphonuclear neutrophils, macrophages, and lymphocytes, and low concentrations of lactoferrin and immunoglobulines (Oliver and Sordillo, 1989). New infections in the dry period are important for several reasons. During the 1st month of lactation, a quarter newly infected in the dry period will sustain a production loss equal to that of a quarter that retains an established infection throughout the dry period (Smith et al., 1968). If the infection persists throughout lactation, proportional production loss would be expected to continue. In addition, at least in herds with a low prevalence of (chronic) infection, most clinical mastitis cases in early lactation are the result of new dry period infections (Eberhart and Buckalew, 1977).

5.1.2. AIMS OF THE DRY COW THERAPY

As it is general in the bovine medicine, dry cow therapy is an intramammary treatment of udder with antibiotics administered at the end of the lactation. Antibiotic treatment at drying off aims at both eliminating the existing IMI and preventing new infections (Neave et. al., 1966). During the dry period, elimination of the infection with antibiotics is more likely than during lactation so that the drug is not milked out, and a higher and more uniform concentration of antibiotics is maintained in the udder. In addition, there are no economical losses due to discarding of antibiotic containing milk (Sandholm and Pyörälä, 1995b).
Experimental evidence suggests that dry cow therapy is effective in controlling IMI due to *Streptococcus agalactiae* and somewhat effective against *Staphylococcus aureus* (Bramley and Dodd, 1984; Dodd, 1983; Eberhart and Buckalew, 1972; Natzke, 1971, 1981; Natzke et al., 1972; Philpot, 1979; Sinkevich et al., 1974; Ziv et al., 1981). Exposure of mammary gland to these contagious pathogens during the dry period is most likely reduced in the absence of regular milking so that therapy at drying off tends to control these pathogens effectively (Oliver and Sordillo, 1988). However, some studies showed that contagious pathogens, especially *S. aureus*, are likely to establish new infections after drying off in those herds where they are prevalent (Eberhart and Buckalew, 1972; Neave et al., 1950; Pankey et al., 1982; Postle and Natzke, 1974; Smith et al., 1966; Smith et al., 1967; Ziv et al., 1981). Neave and Oliver (1962) reported that *S. aureus* could often be isolated from the teat skin after the last milking of lactation, but not from uninfected quarters 28 days later. This suggests that exposure to contagious pathogens is concentrated at the beginning of the dry period but becomes less intense as the dry period progresses. However, elimination of *S. aureus* by therapy is less successful than that of streptococci (Natzke, 1971; Sandholm and Pyörälä, 1995b ; Smith et al., 1967; Ziv et al., 1981;).
Coliform bacteria and streptococci other than *Strep. agalactiae*, which includes primarily *Strep. dysgalactiae* and *Strep. uberis* (but sometimes also *Enterococcus faecalis* and other species of fecal streptococci) are ubiquitous in the cow's environment. Consequently, mammary glands are exposed continuously to environmental mastitis pathogens throughout the dry period, especially in herds in total confinement housing. Schukken et al. (1993b) found, that in low somatic cell count herd the administration of antibiotics at drying off resulted in lower clinical mastitis incidence in the dry period (10 cases for untreated vs. 1 case for treated quarters). The quarters infused with antibiotics had a reduction of minor mastitis pathogens at calving. Williamson et al. (1995) examined the prophylactic effect of a dry-cow antibiotic against *Strep. uberis.* The therapy reduced significantly the incidence of both dry period and post-calving infections. Hassan et al. (1999) noted a marked reduction in the number of infected quarters and clinical mastitis cases caused by *Strep. uberis* and *Strep. dysgalactiae* after dry cow treatment in 2 weeks after drying off. These studies suggest that dry cow therapy can play an important role in the prevention of new infections with these environmental organisms during the dry period.

5.1.3. DRY COW PREPARATIONS

Since the udder is most susceptible to new infections during the first weeks (mostly caused by environmental pathogens e.g. *Strep. uberis*, and maybe contagious pathogens) and last weeks (mostly caused by environmental pathogens including coliform bacteria too) of the dry period (Oliver and Sordillo, 1988; Smith et al., 1985), optimally, the therapy should be extended over the whole dry period. The involuted udder is naturally resistant to Gram-negative microorganisms because of the high concentration of lactoferrin, and the low citrate:lactoferrin molar ratio in secretions inhibit their gowth (Dutt, 1985; Todhunter et al., 1982), so their role in the dry period infections is minor in general. Dry cow antibiotic preparations, therefore, require a good activity against *S. aureus* including β-lactamase producing strains, *Strep. uberis*, *Strep.*

dysgalactiae, *Strep. agalactiae* and, if prophylaxis against summer mastitis is desired , they should also be effective against *Arcanobacterium pyogenes* (Ziv, 1994). Intramammary injectors containing narrow spectrum penicillins (penicillin, cloxacillin, oxacillin, and nafcillin), cephalosporins and spiramycin are therefore widely used.
The dry cow preparations are formulated (vehicles, solvents, pH) to cause minimal tissue irritation, to avoid damaging the secretory tissue and to prevent fibrosis. It is advantageous if the antibiotic is bound to the tissues for an extended period and does not immediately diffuse from the udder into blood. The antimicrobial effect must be long-life, since the purpose is to form a deposit in the milk ducts of the udder from which the antibiotic is slowly released (Sandholm and Pyörälä, 1995b).
The duration of the antibiotic effect can be regulated by pharmaceutical manipulation of the intramammary drugs e.g. precipitating the antibiotic, dissolving it in a slowly absorbing oil or micro-encapsulation.
One significant limitation of antibiotic formulations used for dry cow therapy is the ineffectiveness in preventing new IMI during the periparturient period (Eberhart and Buckalew, 1977; Oliver, 1987; Smith et al., 1985). Boyd et al., (1987) and Oliver and Maki (1987) demonstrated that dry cow antibiotics persisted only for 14 to 28 days after infusion.
In contrast to widely used long-acting intramammaries, Osteras et al. (1991, 1999a), reported the use of short-acting, lactational preparations at drying off. They compared a long-acting and a short-acting injection containing penicillin and neomycin or streptomycin respectively. An injection of short-acting preparation was administered every second day (during eight days) before drying off had a significantly better effect in preventing new infection with *S. aureus* or *Strep. dysgalactiae* in untreated healthy quarters in cows with fewer than 3 infected quarters. This difference in preventive effect was greater in cows with one infected quarter during previous lactation (Osteras et al., 1994). This short-acting therapy resulted better approach for eliminating major pathogens (*S. aureus* in particular) (Osteras et al., 1999a). However, their finding that the use of lactating formula increases the risk of resistance development decreases the value of these otherwise promising results according to the elimination of major pathogens (Osteras et al., 1999b).

5.1.4. SYSTEMIC DRY COW THERAPY

Systemic dry cow therapy may have advantages: better distribution of the suitable drug in the udder tissue which may lead to better cure of IMI (Ziv, 1980a) and avoids new infection which is a possible risk at administration of intramammaries (Boddie and Nickerson, 1986). In the last 10 years some reports were published dealing with systemic dry cow therapy. Bolourchi et al.(1996) found that systemic enrofloxacin or tylosin (a macrolide related to spiramycin) at drying off approached but did not exceed the efficacy of the local treatment with a combination of nafcillin, penicillin and dihydrostreptomycin. Norfloxacin-nicotinate was reported to be effective drug for systemic treatment of *S. aureus* IMI. In the same experiment oxytetracycline showed much lower activity (Soback et al., 1990). However, the promising results of this pilot study could not be confirmed in later experiments. Erskine et al. (1994) published similar findings concerning to the oxytetracycline. In a study with 30,000 IU/kg spiramycin administered intramuscularly on 4 consecutive days at drying off, the bacteriological cure rate of cows with chronic subclinical *S. aureus* mastitis remained below 50 %. Thus the suggested superiority of systemic administration at drying off, compared with conventional intramammary treatment, has not been proven in the practice.
Despite these therapeutic failures, in general the systemic administration of antibiotics at drying off (penicillin; Johansson et al., 1995) or at some weeks before parturition (tylosin; Zecconi et al., 1999) seems to be an effective, supplementary treatment for intramammary therapy of *S. aureus* IMI, which may be advisable for the practice.

5.1.5. THE POSSIBLE ADVERSE EFFECTS OF DRY COW THERAPY

It has been stated that the dry cow therapy may have the following adverse effects (Sandholm and Pyörälä, 1995b):

1.) Discarded meat and milk, if the cow is slaughtered within the withdrawal time or the cow calves prematurely.
2.) A random antibiotic therapy kills the normal bacterium flora of the teat end and teat canal allowing pathogenic and antibiotic-resistant bacteria to colonize the area.
3.) Large-scale use of antibacterials increases selection-pressure to spreading of antibiotic-resistant bacterial strains.
4.) Irritation of teat ends.
5.) Unnecessary treatment of healthy quarters is expensive.

5.1.6. SELECTIVE DRY COW THERAPY

To minimize the adverse effects of antibacterial treatment it has been suggested that only infected quarters or cows are treated at drying off. Poutrel and Rainard (1981) suggested that selective treatment of all cows with at least 1 California Mastitis Test (CMT)-positive quarter at 8 weeks before drying off is the simplest and most economic treatment for herds with a low mastitis infection rate.
According to the antimicrobial drug policy in Nordic countries (Forshell et al., 1996), the effects of selective dry cow therapy were studied. Although the selective dry cow therapy was reported as beneficial compared to no therapy (Osteras and Sandvik, 1996), the authors (Osteras et al, 1991) found that selective dry cow therapy on quarter basis determined from the results of single samples taken 1 to 6 weeks before drying off had given „inadequate" therapeutic response (i.e. new infection in non-treated quarters at drying off) in more than 50% of the cows. Its cause could be that the bacteriological findings from milk can vary from day to day because of intermittent shedding (Mattila, 1985), therefore at least two samples (e.g. 1 month and closely before drying off) could guarantee the adequate specificity. In an other study (Osteras et al, 1999a) evaluating the real efficacy of methods used to identify the infected udders, the geometric mean of the cow composite somatic cell counts (SCC) of the last 5 to 6 months of lactation was the best predictor. However, the treshold value between quarters considered healthy or infected was 200,000/ml, e.g. much lower than generally supposed in the practice. This finding is in good agreement with the earlier observations of Meek et al. (1980).
In addition to the difficulties in diagnosis, a weakness of selective therapy is that ignores infections occurring during and after drying off. Selective quarter treatment (treat infected quarters only) results in a higher new infection rate in the dry period (Browning et al., 1990; Browning et al., 1994)
Selective cow treatment (treat all quarters of any cow infected in one or more quarters) is a preferred concession between selective quarter treatment and blanket therapy (treat all quarters of all cows) (Browning et al., 1994). This is in agreement with the opinion of Sandholm and Pyörälä (1995b): decision as to whether to treat or not has to be made on the basis of the cow, not the quarter. If the cow has had acute or subclinical mastitis caused by contagious pathogens during lactation it is worth treating all the quarters of that cow with dry cow preparations. However, Natzke et al., (1975) calculated that in a 100-cow herd the production gain from prevention of only nine quarters (2.2% of quarters) would return the cost of antibiotic treatment of all cows. In addition, other studies have shown that in low prevalence herds in which selective therapy was used, infection rate was higher at calving than at drying off (Eberhart and Buckalew, 1977; Schultze, 1983). From these consideration, it seems clear that

selective therapy, as compared with complete one, cannot be justified economically in most herds (Eberhart, 1986).

5.1.7. DRY COW THERAPY IN THE PRACTICE

Cows with clinical mastitis are treated according to normal practice before drying off. If mastitis caused by staphylococci early in lactation is a problem in the herd, dry cow therapy can be considered. Dry cow therapy is also recommended for control of contagious mastitis caused by streptococci. Dry cow therapy is recommended for all cows, which have had contagious mastitis during lactation (*S. aureus*, *Strep. agalactiae*, and *Strep. dysgalactiae*). Cows which have had a high milk cell count are also treated. Systematic dry cow therapy is recommended for herds with a high infection rate. Use of germicidal teat dipping during the dry period is also advised for these herds, to reduce the exposure of pathogens on the teat end (Sandholm and Pyörälä, 1995b).

On the other hand, it is important to mention that cows that have had at least one case of clinical mastitis and high geometric mean of SCC in the last 5 to 6 months before drying off should be considered for culling, because they retain a high risk of subsequently having a major pathogen (mainly *S. aureus* and *Strep. agalactiae*) (Osteras et al., 1999a).

Dry cow therapy is also suggested in herds with low somatic cell counts and low prevalence of contagious mastitis pathogens, to minimize the new dry period infections by environmental pathogens which can result a high incidence of clinical mastitis in the early lactation (Eberhart, 1986; Oliver and Sordillo, 1988; Schukken et al., 1993b).

Because continuing exposure to new bacteria during the dry period comes only from the cow's environment, it is reasonable to believe that minimizing exposure to bacterial loads in the environment will reduce a new infection rate (Neave and Oliver, 1962; Smith et al., 1985). Because of the susceptibility to infection in the prepartum period, special attention should be paid to the environment of calving cows (Rendos et al., 1975).

5.2. OWN STUDIES:

BACTERIOLOGICAL RECOVERY AFTER INTRAMUSCULAR OR INTRACISTERNAL SPIRAMYCIN-BASED DRYING OFF THERAPY

5.2.1. INTRODUCTION

Mastitis is defined as an inflammation of the mammary gland resulting from bacterial infection in most of the cases. Intramammary infections (IMI) may be manifested in *clinical* form with visible and palpable changes in the udder and/or in the macroscopic appearance of the milk. In certain cases, however, they cause *subclinical mastitis* with no obvious clinical symptoms, when only the elevation of somatic cell count (SCC) of the milk or the presence of other inflammatory markers can be used to detect the impairment of the affected quarter (Huszenicza and Stollár, 1993; Huszenicza et al., 1997; Sandholm, 1995b).

Both the clinical and subclinical forms of mastitis justify veterinary intervention even if the therapeutic considerations may be different. The *acute form of clinical mastitis* requires immediate treatment, which aims at rapid elimination of the infective agent and thus may decrease the injuries to udder tissue. The animal is brought back to production faster and the spread of infection in the herd is prevented (Pyörälä, 1995b). *Subclinical infections* are usually left untreated during the lactation, and undergone routine therapy at drying off when there is no milk loss due to treatment and the bacteriological efficacy is higher (Craven, 1987).

The species of pathogen causing the actual case of mastitis determines the chance for spontaneous or medicine-driven clinical recovery and of complete bacteriological cure (Pyörälä, 1995b). In certain cases the clinical symptoms may disappear but the quarter remains infected by the pathogen. Thus, clinical recovery is more common than bacteriological cure. The difference between the clinical and bacteriological forms of recovery may be very obvious first of all in *Staphylococcus aureus* mastitis. In 35-70 % of cases this pathogen can survive in the affected milk alveoli, and until the subsequent recurrence of clinical symptoms the continuous elevation of SCC and/or the presence of other inflammatory markers in the milk are the only signs indicating the existence of IMI (Craven, 1987; Owens et al., 1997; Pyörälä et al., 1994, Pyörälä, 1995b; Pyörälä and Pyörälä, 1998). This pathogen can survive also in the dry period, so the use of antibiotics at drying off is a valuable tool in the management of this disease. Intracisternal slow-release preparations containing certain β-lactam antibiotics are the most common drugs of choice, but also some macrolides such as spiramycin can be used for this purpose. Due to its pharmacokinetic properties, spiramycin can be administered not only locally but also in a systemic way, although only limited data are available in the literature on its real efficacy in drying-off therapy.

Prompted by the demand raised by the practice, the aim of the study was to compare the efficacy of systemic (intramuscular) and local (intracisternal) spiramycin-based drying-off treatment in terms of bacteriological recovery in *S. aureus* IMI and the postpartum changes of SCC.

5.2.1.1. Spiramycin in the mastitis therapy

The macrolides form a large group of closely related antibiotics produced mostly by Streptomyces organisms. The macrolides consist of a macrocyclic lactam ring, that entitles to their generic name, and typically two sugar moieties and one amino sugar that attach to the ring. The group of macrolides of the most therapeutic importance are characterized by rings

containing 14 to 16 carbon atoms. Spiramycin belongs to the 14-membered macrolide group. It is a weak organic base with pKa 8.2 and thus changes around as the physiological pH affect its lipid-solubility. Spiramycin interferes with protein synthesis of bacteria by binding to the 50 S subunit of the ribosome. It appears to bind at the donor site, thus, preventing the translocation necessary to keep the peptide chain elongating. The effect is essentially bacteriostatic and confined to rapidly dividing gram-positive bateria and mycoplasmas (Urbán, 1999).
In general macrolides are very suitable drugs for systemic treatment of cows, as they easily penetrate from blood to milk, reaching concentrations several times those of serum, due to ion trapping, even tough lower concentrations can be reached in mastitic milk (Ziv, 1980b). Spiramycin has also been shown to accumulate in macrophages 20 times higher concentration than in the medium (Desnottes and Diallo, 1990). Both the above characteristics are very useful when combating inactive, intracellular (phagocyted) *S. aureus* in microabscesses. Spiramycin has been reported to increase the efficiency of phagocytic system (Desnottes et al., 1988). Other advantage of systemic administration of this drug is the absence of irritation to the udder. However, spiramycin has only bacteriostatic (and not bactericidal) effect, and due to its long-lasting accumulation in milk the withdrawal time is too long to allow to use of spiramycin in lactating cows (Jonsson, 1993). The pharmacokinetic properties of spiramycin support the idea that it may be a potent antimicrobial to eliminate (contagious) gram-positives including *S. aureus* IMI at drying off (Jonsson, 1993; Rhone Merieux, 1991). Spiramycin has been formulated for both the systemic and intracisternal drying off therapy.

5.2.2. MATERIALS AND METHODS

5.2.2.1. Herds and Animals

This study was conducted in three commercial large-scale dairy herds. Fifty-seven, 19 and 24 cows with subclinical mastitis (SCC >500,000 in the last three months of their lactation) were included in the study on farms 1, 2 and 3, respectively, but only those with *S. aureus* IMI were used for the final evaluation. In each herd the cows were housed in free stalls, and were milked twice daily in double herringbone parlours equipped with Alfa Laval milking equipment. Cows were dried off approximately 60 days before the calculated calving dates. During the dry period the cows were housed in separate stalls and about 1 week before the estimated calving date they were moved to the maternity unit where they stayed until postpartum day 5-7. On farms 1 and 2 the post-milking teat dipping and drying-off treatment of cows were used routinely, but farm 3 did not apply these procedures. In accordance with the Hungarian practice, feeding was based on maize silage and alfalfa or grass hay, completed with concentrate depending on the actual milk yield.

5.2.2.2. Sampling and Laboratory procedures

From all udder quarters of the cows aseptic milk samples were collected for bacteriological culture during the last milking out, at drying off and again in the first week after calving. The samples were frozen and stored at –18 ^{o}C until the microbiological examination. From each sample, 0.01 ml was streaked onto the surface of one-fourth plate of Columbia agar (Merck, KgaA, Darmstadt, Germany) containing 5% sheep blood and 0.01% esculin. All the plates were incubated at 37 ^{o}C, and were evaluated after 14 to 16 h and again following an additional 24 h. The colonies were tentatively identified according to their morphology, pigment production, Gram-staining, catalase test and the type of hemolysis produced. The strains initially characterized as staphylococci were tested in tubes for coagulase production to con-

firm the identification as *S. aureus*. For the purpose of the current report all other major and minor pathogens isolated were disregarded.
The SCC of milk was also determined (Fossomatic technology) for each quarter just before drying off, on postpartum days 3-4 and then once a week for further 4 weeks postpartum.

5.2.2.3. Treatments

The cows were randomly divided into four treatment groups. (1) The animals in the first group served as untreated *controls* (number of cows : n_{cow} =21) and no antimicrobials were given to them at drying off. Those in the second and third groups received an intramuscular injection of 30,000 IU/kg spiramycin base (5ml/100 kg; Suanovil^R 20, Rhone Mérieux) (2) in a single dose (*single IM group*; n_{cow} =25) or (3) for 4 consecutive days (*4 IM group*; n_{cow} =30). (4) An intracisternal preparation with 1.2 million IU spiramycin and 100 000 IU neomycin bound to a long acting excipient (Speciorlac^R, Rhone Mérieux) was administered into each of the udder quarters in the last group (*intracisternal group*; n_{cow} =24). All treatments were administered immediately after the last milking out at drying off.

5.2.2.4. Statistical analyses

Statistical analyses were performed using the program SPSS^R (SPSS Version 7.5, 1996, SPSS Inc., Chicago, Illinois, USA). Chi-square tests for non-parametric features, ANOVA and t-test for SCC were used and statistical significance was declared at the 5% level.

5.2.3. RESULTS

S. aureus was isolated in pure culture only from 65 quarters of 38 cows at drying off (the number of *S. aureus* infected cows and udder quarters in the four treatment groups: control: n_{cow} =9, $n_{quarter}$ =11; single IM group: n_{cow} =9, $n_{quarter}$ =14; 4 IM group: n_{cow} =12, $n_{quarter}$ =25; intracisternal group; n_{cow} =8, $n_{quarter}$ =15). The udders of the other animals either (1) were infected by other gram-positive pathogens (mainly by *Strep. dysgalactiae* and *Str. uberis*), (2) the presence of no bacteria could be verified, or (3) also several colonies of contaminating bacteria also grew up. Thus, all of these cows had to be excluded from the current evaluation.
The *bacteriological cure rate* was 18% in the untreated controls. Compared to data of the untreated *controls* and the *single IM group* a quite clear tendency of improved bacteriological recovery rate could be seen in the *intracisternal* and *4 IM groups.* However, only the quarter-based difference between *4 IM* and *single IM* groups was significant ($P<0.05$). Even the highest bacteriological cure rate of *S. aureus* infected quarters observed in the *4 IM group* remained below 50%. The single intramuscular administration of spiramycin seems to be completely ineffective for the elimination of *S. aureus* IMI (*Table* 25, *Fig. 13*).

Table 25
The bacteriological cure rate of cows infected with *S. aureus* at drying off.

Treatment	Quarters			Cows		
	Infected n	Cured[#] n	Recovery rate %	Infected n	Cured[##] n	Recovery rate %
Untreated control	11	2	18	9	2	22
Treated with spiramycin						
Intracysternal	15	6	40	8	3	37
Intramuscular, single	14	2*	14	9	2	22
Intramuscular 4 times	25	12*	48	12	3	25
All spiramycin treated	54	20	37	29	8	27

Cured: If from samples taken after calving *S. aureus* was not re-isolated *from that quarter*[#] or *from any udder quarters of that individual*[##].
*Between these values there is a significant difference (P<0.05).

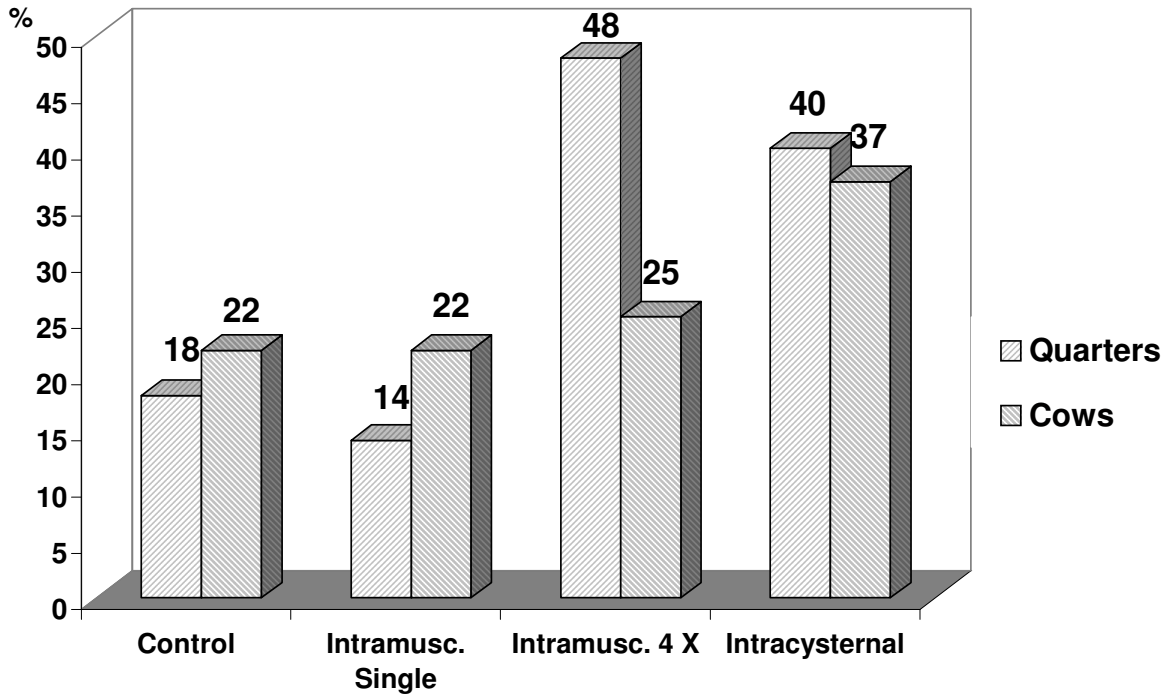


Fig. 13. The bacteriological cure rates of quarters and cows infected with *S. aureus* at drying off

At drying off the *group mean of SCC* was over 1 million/ml in each group and no significant difference was seen among them. In accordance with the bacteriological cure rates observed, the group means of SCC were about 600,000/ml in the *intracisternal* and *4 IM groups* and about 1,000,000/ml in the other groups in the 1st week after calving. These 1st-week values were followed by a continuous decrease in all groups to the range between 300,000 - 500,000 (*intracisternal* and *4 IM groups*) or between 500,000 and 800,000 (in other groups). Due to the relatively high rate of overlapping values, however, no significant inter-group differences could be demonstrated.

5.2.4. DISCUSSION

In this experiment single and repeated intramuscular spiramycin treatment at drying off were compared with the local (intracisternal) spiramycin+neomycin therapy as well as with untreated controls. The only existing intracisternal preparation used also in this study contains not only spiramycin but also an aminoglicoside (neomycin). Under *in vitro* conditions the neomycin is usually active against *S. aureus*. However, in the mammary gland most of these bacteria are located deep in udder tissue in damaged milk alveoli surrounded by fibrotic tissue. Some of them may be phagocyted by neutrophyl granulocytes, but they can survive intracellularly in L form within the lysosomes of these cells. Due to these conditions aminoglicosides – such as neomycin – are unable to reach and kill them (Jonsson, 1993; Sandholm et al., 1990).

S*ingle IM* treatment was also chosen as a practical regime. The *4 IM* way of administration was based on the assumption that longer therapy may result in higher cure rate (Sandholm, 1995a). Spiramycin was administered in the normal dose suggested by the manufacturer (30,000 IU/kg).

The *single IM* treatment produced the same very low quarter-based bacteriological cure rate (14%) as seen in untreated *controls* (18%). In accordance with these bacteriological findings the SCC remained elevated (> 400,000/ml) in the early weeks of the subsequent lactation, indicating the presence of chronic IMI and subclinical mastitis in most of these cases. When administered intramuscularly, spiramycin in dose of 30,000 IU/kg can produce a 24 hours drug level above the in vitro MIC of *S. aureus* in milk (Ziv, 1976) which seems, however, to be too short for the elimination of this pathogen. Due to its short effect, this treatment must also have failed to prevent new IMI during the dry period. This idea is supported by the observation that the *4 IM treatment regime* resulted in a significantly higher quarter based cure rate (48%). The about 40% of bacteriological cure rates in the *intracisternal* and *4 IM groups* were almost the same.

If more than one udder quarters of a cow proved infected at drying off (75 % of the cows in 4 IM group and 50 % cows in IC group), in most of the cases at least one of their quarters remained uncured after calving. Thus the individual-based evaluation of bacteriological cure rates gave even poorer results than the quarter-based evaluation. These findings suggest that antibacterial therapy has questionable value in cows with more than one infected udder quarters.

As a cause of the moderate effectiveness of these therapies, we should consider, that following the local (intracisternal) or systemic (intravenous, intramuscular) administration of antimicrobials the bacteriological recovery rate is affected not only by the *in vitro* susceptibility of the mastitis pathogen to the injected ingredient but also by several other factors. The pharmacokinetic properties of the antimicrobial, the species of the pathogen, as well as the acute or subacute-chronic character, the location and pathological consequences of IMI are known as the most important influencing factors (Pyörälä, 1995b). Staphylococcal mastitis is typically chronic. In such cases the staphylococci appear inside neutrophils and macrophages. Unlike most bacteria, these can resist phagocytosis and remain alive. Inside the cell the low pH interferes with the effect of antibacterials. In addition, the metabolic status of the microbes inhibits antibacterials being effective. The logarithmic phase of bacterial growth is usually complete by the time treatment begins. In chronic staphylococcal mastitis, the alveoli are involuted, surrounded by fibrous capsule and inside they contain neutrophil granulocytes and bacteria (Pyörälä, 1995a). As a consequence, very low cure rates are reported (maximum about 50%; Craven, 1987) when the follow-up bacteriological assessment is made 3 weeks

after therapy (Pyörälä and Pyörälä, 1998). Due to the 20-25% self recovery rate, the net effect of antibiotics on *S. aureus* is significantly lower than 35-50 % (Sandholm et al., 1991).

The environment of staphylococci in microabscesses is different from the milk, which is the only compartment where spiramycin can reach concentration above the MIC of bacteria for a longer period (Franklin et al., 1986). The intercellular (interstitial) antibacterial effect of the macrolides, however, been shown to be poor (Pyörälä, 1995a). This may be one of the causes of surviving of *S. aureus* during the dry period in the udder.

All of our treated cases were chronic, and expected to be resistant most of the antibacterial therapies. Our observations verifying the relatively low efficacy of any of these treatments against *S. aureus* IMI show a close agreement with the results of others (Bolourchi et al., 1996; Craven, 1987), and do not support the earlier expectations (Jonsson, 1993; Rhone Merieux, 1991;) concerning the outstanding value of this antimicrobial in the management of *S. aureus* mastitis.

5.2.5. CONCLUSION

It can be concluded that the *single IM* treatment regime is not an effective way of drying-off therapy against *S. aureus*. If the intramuscular spiramycin administration was continued for 4 days, or if this ingredient was injected intracisternally in the form of a preparation developed for drying-off therapy, the efficacy was higher but remained below 50 %. Due to the high cost of the repeated intramuscular treatment regime there is no reason to give extra preference to this therapy in the practice.

6. CHAPTER 3

BOVINE MASTITIS CAUSED BY THE ALGA *PROTOTHECA ZOPFII*

6.1. REVIEW OF THE MICROBIOLOGICAL, PATHOLOGICAL, AND CLINICAL ASPECTS OF BOVINE MASTITIS CAUSED BY THE ALGA *PROTOTHECA ZOPFII*

6.1.1. INTRODUCTION

Besides several bacteria and some other microorganisms, unicellular alga of *Prototheca zopfii* also can cause mastitis in dairy cows. The sporadic form of algal mastitis has been diagnosed almost worldwide. The endemic form of this disease, however, occurs mostly in the wet and humid tropics, where the climatic conditions may be more advantageous for the rapid multiplication of this pathogen in the environment (Costa et al., 1996b, 1997, 1998). In the temperate zones of the world, this form may occur in geographical areas with a relatively high humidity (Baumgärtner, 1997; Frank et al., 1969; Hodges et al., 1985; McDonald et al., 1984b; Spalton et al., 1985). As far as we know, however, an epidemic of algal mastitis has not been reported on large-scale dairy farms in countries with relatively dry continental climatic conditions. In Hungary no cases of algal mastitis have been reported until 1998-1999. However, in these years, we detected several cases of *Prototheca* mastitis in 32 dairy herds. In our routine diagnostic work we have been surveying the outbreaks of algal mastitis.

6.1.2. TAXONOMIC CLASSIFICATION AND BIOLOGY

Eukaryotic algae are regarded as the most primitive representatives of the plant kingdom. During phylogenesis, one group of unicellular algae lost its green pigments and changed over to heterotrophic nutrition. As early as the 1880's, Zopf and Kühn cultured until then unknown microorganisms from a mucous secretion of a linden-tree. The first morphological and physiological characterization of these isolates was made by Krüger in 1894 (Krüger, 1894), who distinguished two species by the names *Prototheca zopfii* and *P. moriformis.* Since then oher representatives of *Prototheca* genus have been described, and some of them have been attributed a pathogenic character. *P. stagnora* cannot grow at 37 °C, so in birds and mammals its pathogenic role is improbable (Pore, 1985). The known human pathogen *P. wickerhamii* may primarily cause malignant, chronic skin lesions, but in immunocompromised hosts it may even give rise to systemic disease (Carey et al., 1997; Kim et al., 1996; Mohaber et al., 1997). In dogs, and less often in cats and other species of animals, the above *Prototheca* species can produce granulomatous skin lesions like those seen in humans. In dogs *Prototheca* often affects the eye, causing retinopathy leading to blindness, or produces a systemic disease with a usually fatal outcome (Blogg and Sykes, 1995; Dillberger et al., 1988; Ginel et al., 1997; Moore et al., 1985). *P. moriformis,* characterized by Pore (1985) as a distinct species, can be detected primarily in the environment, but has also been cultured from bovine milk samples (Pore et al., 1987). The most frequent animal disease of algal origin is, however, the bovine mastitis caused by *P. zopfii* (synonyms: *P. trispora, P. segbwema*) (Seffner, 1987).
The sexual reproduction of algae belonging to the genus *Prototheca* is not known. Their asexual reproduction involves the production of cells of characteristic shape and size. During ontogeny, a thick-walled, large (10–30 μm) cell of oval or spherical shape develops (sporangium). This forms 2 to 16 or perhaps more aplanospores (endospores, sporangiospores) by division. The wall of this mulberry- or morula-like parent cell ruptures, releasing endospores that enlarge and repeat the reproductive cycle. In 1-3 % of sporangia, 2–3 thick-walled resting cell stages (hypnospores, dauer Zell) are formed (Pore, 1985). The cell wall is distinctive in that it possesses a trilaminar layer of sporopollenin and contains neither chitin nor cellulose. Some species may develop a mucopolisaccharide capsule, which is common for green algae

and higher plants (Dodge, 1973). Cells are always non-motile. All species of *Prototheca* utilize glucose as a carbon source. They can equally use inorganic N salts (ammonium salts) and proteins as nitrogen sources. All species require oxygen and thiamine for growth (Pore, 1985).

6.1.2.1. Cell culture and identification

Media containing glucose (e.g. Sabouraud's dextrose agar) are the most suitable for culturing *P. zopfii* (Costa et al., 1997; Pore et al., 1983). Contaminating bacteria and fungi might overgrow *Prototheca.*To suppress bacterial contaminants, 100 mg/L chloramphenicol may be added to the medium (Costa et al., 1997). *Prototheca Isolation Medium* (Pore, 1973) and *Prototheca Enrichment Medium* (Pore et al., 1987) containing inhibitory substances (5-fluorocytosine) can be used for the selective isolation of *Prototheca* and *Chlorella* algae. Selective culturing of *Prototheca zopfii* is also possible on media with pH 5.1, containing acetate as the only carbon source (Pore et al., 1983). Stereomicroscopic observation may help to identify alga colonies after 24 h incubation (Pore et al., 1987).
Members of the genus *Prototheca* must be differentiated from yeasts. When cultured aerobically on Sabouraud's agar at 37 °C for 48 h, *P. zopfii* forms flat, colorless or whitish colonies approx. 2 mm in diameter with irregular margins, granular surface and a compact central protrusion. Yellowish-white colonies of wax-like consistency 5–7 mm in diameter are seen after longer incubation. The organism forms small, grey, dull colonies with irregular surface on blood or blood-esculine agar. *P. wickerhamii* forms regular hemispherical colonies with a smooth surface, while *P. stagnora* forms flat colonies with entire margins and a relatively smooth surface (Seffner, 1987). Rapid differentiation of *Prototheca* from *Candida* species can be achieved by the so-called aggregation test (Müller, 1988) which makes use of the hydrophobic character of *P. zopfii*. A susceptibility test for ribostamycin (60 µg/disc) is suitable for differentiating between *Candida* and *Prototheca* species. Unlike resistant budding yeasts, *Prototheca* isolates show an inhibition zone 25–28 mm in diameter after incubation (Casal and Gutierrez, 1986). By light microscopy, the mulberry-like sporangia containing endospores can be visualized in smears made from the cultures (or from the sediment of milk samples) with simple staining procedures and with 400-fold magnification. The sporangia of *P. zopfii* are usually ovally elongated or spherical and 15–30 µm in diameter. Released endospores with a starch-granulated cytoplasm are oval and 8–16 µm in diameter. All cell stages of *P. wickerhamii* are spherical and average cell size is about half that reported for *P. zopfii.* Capsule production, detectable by Indian ink staining (the capsule does not stain), occurs in *P. stagnora* and *P. moriformis* (identified by Pore as a distinct species) (Pore, 1985; Seffner, 1987). (*Table 27*)
The differentiation of *Prototheca* species is facilitated by carbohydrate assimilation tests as well as antimicrobial sensitivity tests with neomycin (*P. zopfii* is sensitive) (Casal and Aroca, 1995) and clotrimazole (*P. zopfii* is resistant) (Aalbaek et al., 1988). Some differentiating properties of three *Prototheca* species are presented in *Table 26* (Blaschke-Hellmessen et al., 1985). Studies of *P. zopfii* strains isolated from different sources revealed the existence of three types within the species (*Table 27*) (Blaschke-Hellmessen et al., 1984; Blaschke-Hellmessen et al., 1985; Schuster and Blaschke-Hellmessen, 1983). Type II, characterized by intensive glycerol utilization and delayed or missing galactose assimilation, has been cultured from most cases of mastitis (Seffner, 1987; Wilhelm et al., 1992). Type I and III have not been reported previously as mastitis pathogens. (*Table 27*)

Table 26
Properties suitable for the differentiation of the three *Prototheca* species (after Blaschke-Hellmessen et al., 1985)

	P. zopfii	*P. wickerhamii*	*P. stagnora*
Cell size (µm)	7–30	4–10	7–14
Assimilation			
Glucose	+	+	+
Galactose	(+)	+	+
Sucrose	–	–	+
Trehalose	–	+	–
n-Propanol	+	–	–
Glycerol	+	+	+
Capsule production	–	–	+
Growth at 37 °C	+	+	–

Table 27

Differentiation of the three types of *P. zopfii* (after Blaschke-Hellmessen et al.,1985)

	Assimilation		pH tolerance	NaCl tolerance	Cell shape
	Galactose	Glycerol			
Type I large or very large cells	++	+++	2.4–9.5	4% NaCl	spherical and oval
Type II moderately large cells	(+)	+++	2.1–10.5	6% NaCl	spherical and oval
Type III moderately large or large cells	+	+	4.0–10.5	4% NaCl	spherical

6.1.2.2. Prevalence

Algae belonging to the genus *Prototheca* are widespread in nature and occur all over the world, primarily in humid habitats rich in organic materials. They have been detected in rivers, drinking water sources, communal and slaughterhouse sewage, wet soil, manure and the faeces of cattle, pigs and certain laboratory animals (Anderson and Walker, 1988; Pore et al., 1983; Pore and Shahan, 1988). The possibility of mechanical transmission by insects and rodents cannot be disregarded (Pore and Shahan, 1988; Pore et al., 1983). In natural waters deficient in organic matter these algae are present only temporarily (Pore et al., 1983).
The presence of *Prototheca* algae in faeces of animals was related to consumption of contaminated feeds. Faecal shedding of these algae ceased when the experimental animals started to receive *Prototheca*-free fodder (Pore and Shahan, 1988). Resistance of *Prototheca* to digestive processes in the gastrointestinal tract is attributed to the sporopollenin content of the cell wall. Algae can become especially abundant in muddy or wet outdoor runs, paths where animals are driven, resting areas and pastures contaminated with slurry (Anderson and Walker, 1988; Baumgärtner, 1997; Costa et al., 1996c; Costa et al., 1997; Pore et al., 1983; Schuster and Blaschke-Hellmessen, 1983). Calves fed with *Prototheca* containing mastitic milk may be a major source of environmental contamination (Costa et al., 1997). Leftovers of wet feeds

rich in starch, oligosaccharides and disaccharides (e.g. potato pulp) are also considered to be a medium for multiplication (Baumgärtner, 1997; Pore and Shahan, 1988). The presence of *Prototheca* algae has been detected in milking equipment, in its pipelines, and on teat cup rubbers. The organism even survived routine disinfection procedures with a chlorine solution (Anderson and Walker, 1988; Costa et al., 1997). In surveys of prevalence in dairy herds, *Prototheca* species were found to occur in environmental samples even in dairy herds in which algae had not been isolated from cows with mastitis (Anderson and Walker, 1988).

6.1.3. *PROTOTHECA* AS A CAUSE OF MASTITIS

6.1.3.1. Occurrence

Since the first description by Lerche (Lerche, 1952) mastitis caused by *P. zopfii* has been reported worldwide, from temperate and tropical climatic zones as well. Sporadic cases of *Prototheca* mastitis have been found in Europe (Aalbaek et al., 1988; Goudswaard, 1977; Lagneau, 1996; Schlenstedt et al., 1997; Spalton, 1985), North-America (Dion, 1982) and Asia (Furuoka et al., 1989; Katoch et al., 1997; Kuttin et al., 1986; Taniyama et al., 1994). In addition, there are several reports about endemic *Prototheca* mastitis with high numbers of affected cows, mostly from geographical areas with relatively high humidity and/or temperature, e.g. from climatic conditions, which are known to be advantageous for the rapid multiplication of this pathogen in the environment (Baumgärtner, 1997; Costa et al., 1996b, 1998; Frank et al., 1969; Hodges et al., 1985; Linquist, 1981; McDonald et al., 1984b). Although available international references report numerous cases of mastitis caused by *P. zopfii*, the real incidence of this disease can be estimated to be much higher, because of the possible misidentification of *P. zopfii* as a yeast or failure to growth under routine microbiological conditions (Gedek and Weber, 1978; Spalton, 1985).

6.1.3.2. Clinico-pathological features

Mastitis caused by *P. zopfii* is most often recognized as a chronic, symptom-less process with very high somatic cell count (over 10^6/ml); however acute, clinical mastitis may also occur (Anderson and Walker, 1988; Costa et al., 1996a; Schuster and Blaschke-Hellmessen, 1983).
The tissues of the lactating udder are highly susceptible to *P. zopfii* infection. Under experimental conditions, infusion of 40–480 colony forming units (CFU) of *P. zopfii* into the udder through the teat resulted in mastitis in 100 % of the inoculations. Five days after the experimental infection the pathogen could be cultured from the milk samples in an amount of 20,000–50,000 CFU/ml (McDonald et al., 1984b).
In acute cases, the sero-purulent form of mastitis can be observed with large numbers of alga cells in milk, in the alveolar epithelial layer, in macrophages, and in the interstitium. Pronounced proliferation of interstitial connective tissue and concurrent atrophy of alveoli (Frank et al., 1969) characterize chronic cases. As compared to mastitis of bacterial origin, in mastitis due to algae infiltration of the udder tissue with mononuclear cells is more pronounced (Frank et al., 1969; McDonald et al., 1984b). Proliferative processes associated with microgranuloma formation are also considered characteristic (McDonald et al., 1984b; Schönborn and Seffner, 1977). The techniques most suitable for the selective visualization of algae in histological sections include the periodic acid–Schiff (PAS) reaction, Gömöri's silver impregnation stain, and Gömöri's methenamine-silver stain (Frank et al., 1969; McDonald et al., 1984b; Taniyama et al., 1994). Immunofluorescence enables species identification of alga cells in histological sections and cultures (Sudman and Kaplan, 1973). By electron microscopic examination, alga cells phagocytosed by macrophages showed different degrees of degeneration. Ultrastructural examinations did not show the presence of *Prototheca* cells in alveolar epithelia

(Cheville et al., 1984). Both sporangiospores and sporangia have been found, suggesting that intracellular proliferation may have been responsible for the failure to overcome the infection (Jensen et al., 1998)
Systemic symptoms are usually absent, and in most cases even local signs are mild. The most common clinical sign is the changed appearance of milk, which becomes watery and contains flakes and lumps. However, in addition to the pronounced decrease in milk production, in most cases only the prolonged elevation of the SCC indicates that a quarter is infected (subclinical mastitis) (Bergmann, 1993; Costa et al., 1996a, 1996c). Based on its clinical characteristics *Prototheca* mastitis cannot be distinguished from the cases of mastitis of bacterial origin.

6.1.3.3. Epidemiological aspects

P. zopfii can be regarded primarily as a so-called environmental pathogen. When the organism is widespread on a farm, the cows of that herd are subject to increased risk of infection (Anderson and Walker, 1988).. The occurrence rate is higher in herds kept in total confinement system with no pasturing or in a muddy, faeces contaminated pen (Costa et al., 1996c; Costa et al., 1998). The defective pre-milking hygiene of teats plays a major role in the casuality (Costa et al., 1996c). Costa et al.(1998) found a higher occurrence of *Prototheca* mastitis in herds that were not fed immediately after milking.
Highly contaminated, spoiled feed may also be the source of large numbers of *P. zopfii* in the farm environment, and may be responsible as well for the endemic form of mastitis (Baumgärtner, 1997; Pore and Shahan, 1988).
A higher environmental temperature and humidity provide highly suitable conditions for the multiplication of this pathogen. In the summer period – with the above weather conditions in Brazil - Costa et al. (1998) reported significantly higher incidence of new infections than in winter-time.
Cows in the first few weeks of lactation seem to be especially susceptible to natural infection (Costa et al., 1996a, 1997; Frank et al., 1969; Shahan and Pore, 1991). Although mixed infection with bacteria (mainly *Staphylococus aureus, Streptococcus spp*.) can occur, a synergism between *P. zopfii* and other udder pathogen bacteria has not been proven (Schlenstedt et al., 1997). Immunosuppressive therapy predisposes the *Prototheca*-caused infections and may lead to disseminated disease (Taniyama et al., 1994; Wilhelm et al., 1992). In a retrospective case control study of 248 *Prototheca* mastitic cows the age/parity of cows, previous mastitis cases and especially antimicrobial pretreatment of udder quarters were found to increase the risk of protothecal mastitis (Tenhagen et al., 1999).
Contagious (animal-to-animal) transmission probably occurs during milking (Costa et al., 1997; Spalton, 1985).
As a summary, it can be stated that although *Prototheca* mastitis may occur in well-managed herds, its prevalence is sporadic. Only when there are poor housing conditions and poor milking hygiene as well as highly contaminated fodder is the disease likely to become endemic (Baumgärtner, 1997).

6.1.3.4. Therapy

In antimicrobial susceptibility tests performed *in vitro*, the majority of *P. zopfii* strains proved to be moderately susceptible to a few antibiotics and some fungicides (polymyxin B, gentamicin, nystatin, amphotericin B) (McDonald et al., 1984a; Shahan and Pore, 1991; Van-Damme, 1983). In human medicine, high dose administration of amphotericin B or fluconazole has been reported as an effective therapy against *Protothecal* infections (Carey et al., 1997; Kim et al., 1996). Because of its extremly high costs and because of veterinary public health considerations, this treatment procedure is not applicable in dairy cows. Only a tempo-

rary decrease in severity of clinical signs and degree of alga shedding could be achieved by routine use of various antimicrobials in dairy cows (Bergmann, 1993; Costa et al., 1996a; Hodges et al., 1985). Thimerosal resulted in 100 % microbiological cure. However, due to the irreversible damage to udder tissue caused by the long-lasting algal infection, milk production remained low even after the pathogen had been completely eliminated (Costa et al., 1996a).

6.1.3.5. Prevention

P. zopfii mastitis is considered as one of the most difficult forms of this disease to prevent and control (Kirk, 1991). The principles of prevention are the same as those usually followed for other environmental pathogens (Anderson and Walker, 1988; Hodges et al., 1985). It is of utmost importance to prevent contamination of the teats with faeces or wet litter during the first 25–30 minutes after milking, when the relaxed sphincter of the teat canal fails to prevent ascending infection. To achieve this goal, favourable hygienic status and regular cleaning of driving ramps are of crucial importance, as is offering freshly served, palatable feed to animals after milking to prevent them from lying down immediately. The availability of dry bedding is also important. Stagnant leftovers of carbohydrate-rich feeds with a high moisture content must be removed regularly. In severely infected dairy herds, systematic cleaning with high-pressure hot water or spraying with algicides is essential (Baumgärtner, 1997).
Adequate pre-milking hygiene is one of the most important preventive measures. Post-milking teat dipping with disinfectants is an effective way to diminish potential animal-to-animal transmission. Properly functioning milking machines are indispensable to avoid injuries of teat end and teat canal, which predispose to *Protothecal* udder infections (Baumgärtner, 1997; Costa et al., 1998).
Infected cows are known to have a major role in maintaning *Prototheca* contamination of the environment (Costa et al., 1997). In the case of *Prototheca* mastitis, the antimicrobial defence mechanism of the udder cannot be expected to eliminate the pathogen (Van Veen and Kremer, 1992). *Prototheca* algae can survive for a long time in the udder of dry cows (Dion, 1982; Schlenstedt et al., 1997). For this reason, widespread microbiological monitoring should be conducted in infected herds. After their identification, infected animals have to be removed immediately. Separate housing and milking and the early culling of infected individuals semm to be effective in the herd health management of this disease (Anderson and Walker, 1988; Baumgärtner, 1997; Costa et al., 1996a, 1996c; Frank et al., 1969; Hodges et al., 1985; Seffner, 1987).

6.2. OWN STUDIES

PROTOTHECA ZOPFII MASTITIS IN HUNGARIAN LARGE SCALE DAIRY HERDS

6.2.1. INTRODUCTION

In autumn of 1997, a yeast-like microorganism was cultured from some mastitic milk samples collected for an udder health control programme. This pathogen was subsequently identified as the unicellular alga of *P. zopfii*. Since then, the same organism has been isolated from more than 50 large-scale farms on at least one occasion (*sporadic form*); the accumulated outbreak of this masitis has been observed in 6 of these farms (*endemic appearance*). Three of these dairy herds with a high incidence of algal mastitis were closely studied (*farm survey).* As a third step the epidemiological character of this infection was followed on one of the farms (Herd 3.; *epidemiological study*).

6.2.2. MATERIALS AND METHODS

6.2.2.1. The farm survey

Each of the three farms involved in this survey had 200–300 Holstein-Friesian × Hungarian Red Spotted crossbred cows kept in a loose housing system with adjoining corrals. The cows were milked twice a day in double herringbone milking parlors with Alfa Laval™ milking equipment. In the *Herds 2* and *3* a 1% solution of an udder disinfectant containing organic iodine (Hygienius®, Alfa Laval™) was used regularly for post-milking teat dipping, whereas in *Herd 1* this procedure had been stopped a few years earlier for economic reasons. As a part of the regular farm management, in all herds individual bulk milk samples had been collected once a month for many years from each cow to check the somatic cell count (Fossomatik technology, *Hungarian Herd Recording Ltd., Gödöllő*). As is usual in this area, no pasturing was available for the cows. They were fed with ensilaged maize and alfalfa products, alfalfa and grass hay, which were complemented with cereals and vitamin and mineral premix. In the *Herds 1* and *2* wet sugar-beet chips also were given in the autumn.
Altogether 73 mastitic cows were involved in the *farm survey*. After the clinical examination, aseptic milk samplews were collected rom each quarter for microbiological investigations. If the quarter proved to be infected, it was re-sampled again several times 4 to 5 weeks apart during a 12-18-months long period. Simultaneously, the SCC was also checked. The SCC data of the previous lactation were available for retrospective evaluation. If cows dried off, the sampling procedure was re-continued after calving. Also feed (maize silage, wet sugar-beet chips), faecal and bulk milk samples (only in *Herd 3*) were taken for microbiological investigations.

6.2.2.2. Microbiological investigations

For *isolation of P. zopfii* from milk, 0.1 ml of each milk sample was streaked onto Sabouraud's agar plates (art. No.:1.05438, Merck KgaA, Darmstadt, Germany) supplemented with yeast extract and onto 5% sheep blood agar medium (art. No.:1.05450, Merck KgaA, Darmstadt, Germany). The plates were incubated under aerobic conditions at 37 °C for at least 48 h. The pure cultures of colonies were identified according to the recommendations of Pore (1985) and Blaschke-Hellmessen et al. (1985). In *Herd 3* the milk samples taken from quarters of 15 alga-infected cows were cultured also for *mycoplasmae* on modified Hayflick

medium (Whitford et al., 1994). Feed and faecal samples were cultured on Sabouraud's agar plates containing substances (such as 5-fluorocytosine and chloramphenicol) to inhibit fungal and bacterial growth (Costa et al., 1997; Pore, 1973).

6.2.2.3. Histopathology

In the *on-farm survey phase* three alga-infected cows were slaughtered and their udders were subjected to gross and histopathological examination. For histopathology, pieces from different parts of the udder and supramammary lymph nodes were fixed in 5% formaldehyde solution (pH 7.2, buffered with NaH_2PO_4 and NaOH). Then frozen and paraffin-embedded sections were made, which were stained with haematoxylin and eosin (for general examination), as well as with periodic acid-Schiff (PAS) reaction (for selective detection of *Prototheca* cells).

6.2.3. RESULTS

6.2.3.1. Microbiology

During the 2-year study period , 223 *P. zopfii* infected cows were identified in 32 herds. They accounted for 1.6 % of the total 14.000 mastitic milk samples. Following years algae were isolated from several cows of more than 50 dairy herds, and a slight increase was noticed in the rate of *P. zopfii* positive milk samples. In 1999 and in the first 8 months of 2001, the numbers of examined mastitic milksamples were 7125, and 6006, respectively. From these samples pathogens were detected in 4202 and 3229 cases, respectively. From 2% and 4.5% of these bacteriologically positive samples *P. zopfii* was isolated as a pathogen in 1999 and 2001, respectively. (The year 2000 was not evaluable due to the reorganization of routine diagnostic work.)

The isolates grew poorly on blood agar. Intensive growth was seen on Sabouraud's agar. After 2-day incubation, the colonies were white, flattened, slightly protruding, approx. 2–3 mm in diameter, had irregular margins and a characteristically rough surface (*Fig. 14*). 3 strains produced hemispherical colonies with a less coarse surface (*Fig. 16*). In wet smears sporangia representing different segmenting stages (*Fig.15* and *Fig. 17*) were 15–25 µm in diameter. Cell wall remnants of ruptured and emptied sporangia were easily visible after PAS staining (*Fig. 18*). These strains intensively assimilated glucose and glycerol, did not utilize sucrose, trehalose and, with the exception of three strains, galactose. The strains grew but did not produce a capsule at 37 °C. According to Pore (1985) and Blaschke-Hellmessen et al. (1985), strains tolerating pH 2.1 and NaCl concentration of 6.0% (all the isolates but three) and producing mostly ovally shaped cells can be classified as type II of *P. zopfii* (*Fig. 15*.). The further 3 strains with different colony-morphology assimilated glycerol and galactose with moderate intensity, had lower salt and pH tolerance (pH 4.0, NaCl concentration: 4.0%), and its cells were spherical. On the basis of these properties those strains were classified as *P. zopfii* type III (*Fig. 17*).

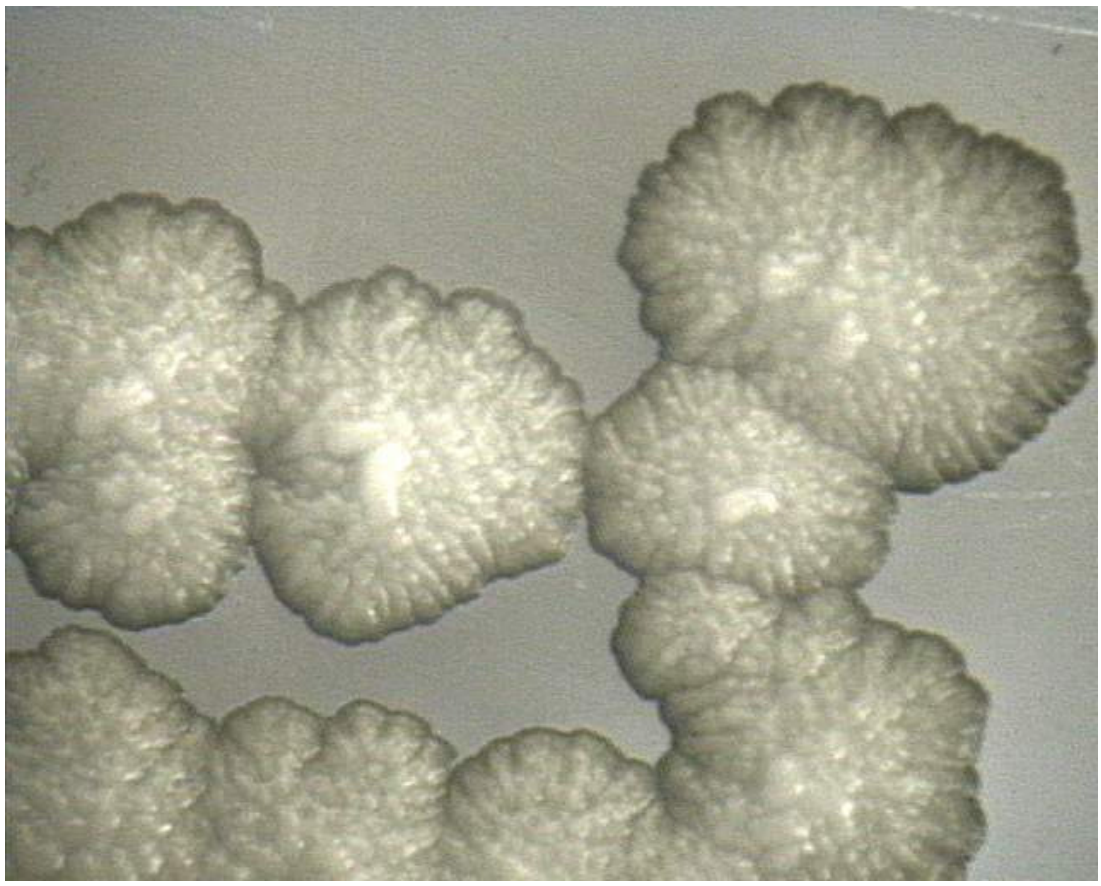


Fig. 14. Colonies of *Prototheca zopfii type II* grown on Saboraud-dextrose agar for 72 hours at 37 C, ×10

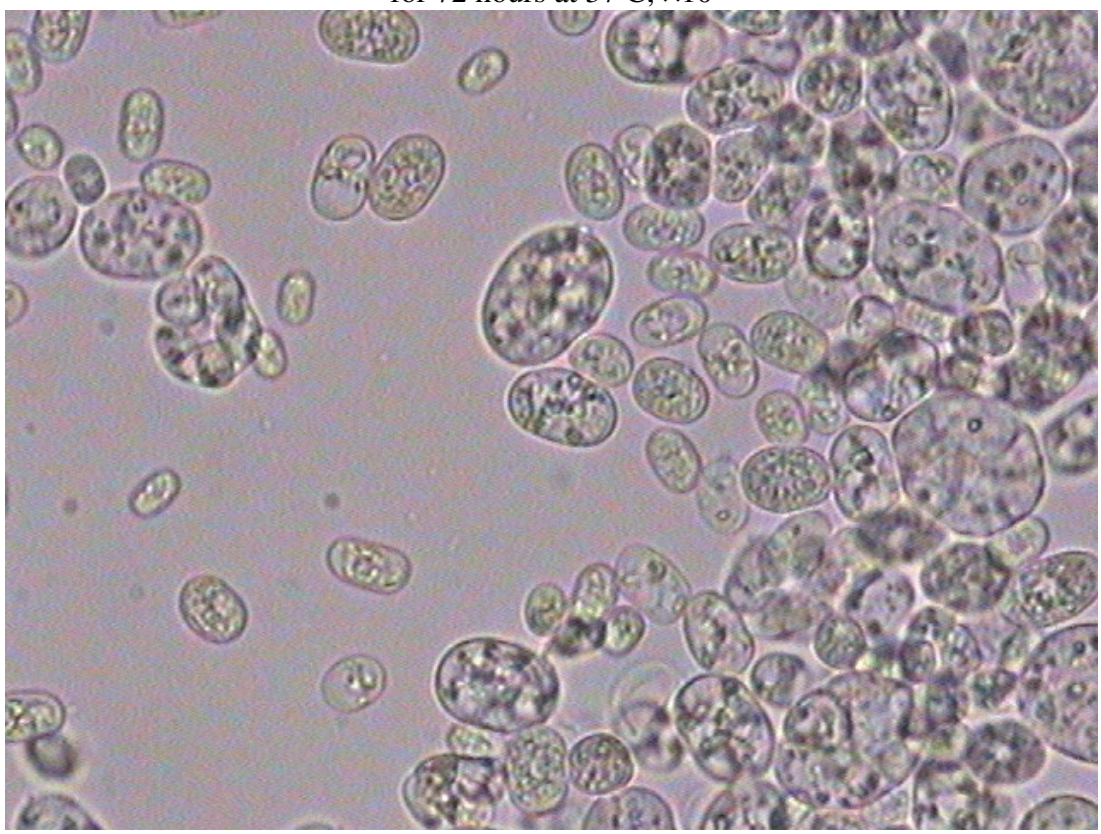


Fig. 15. Sporangia and endospores of *Prototheca zopfii type II,* 48 hours culture, native, wet prep., ×400

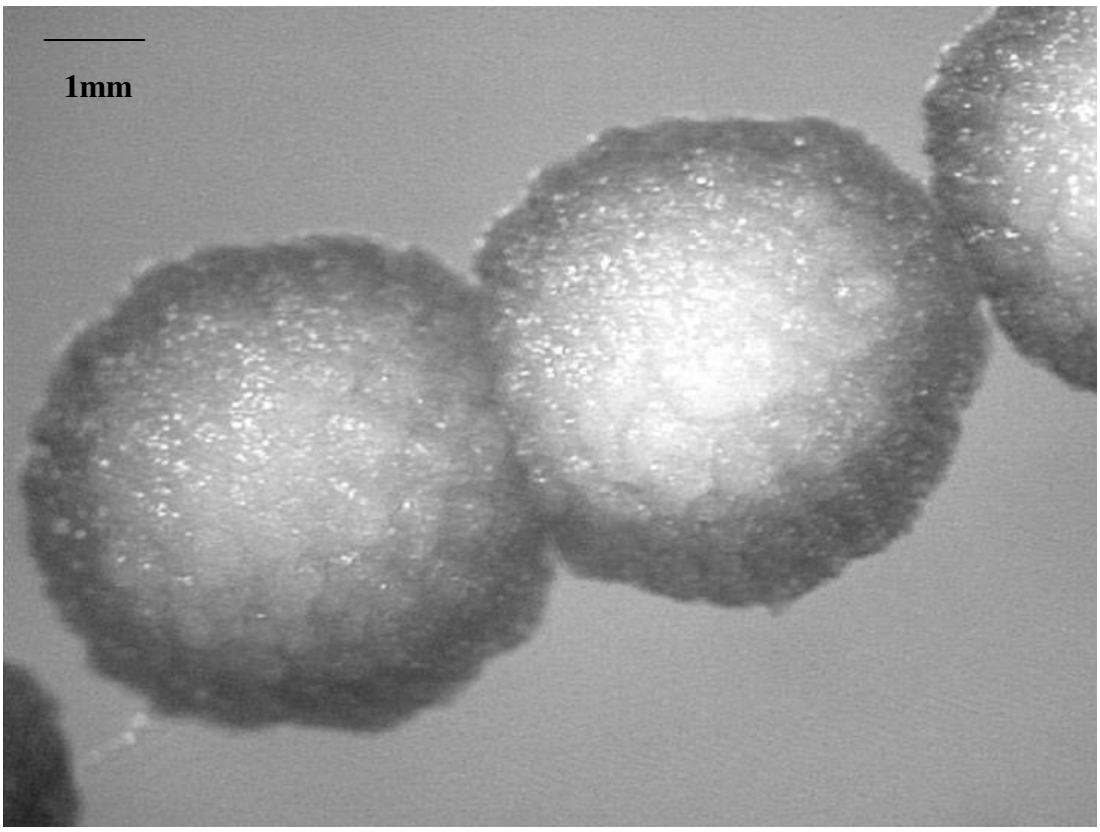


Fig. 16. Colonies of *Prototheca zopfii type III* grown on Saboraud-dextrose agar for 72 hours at 37 C, ×10

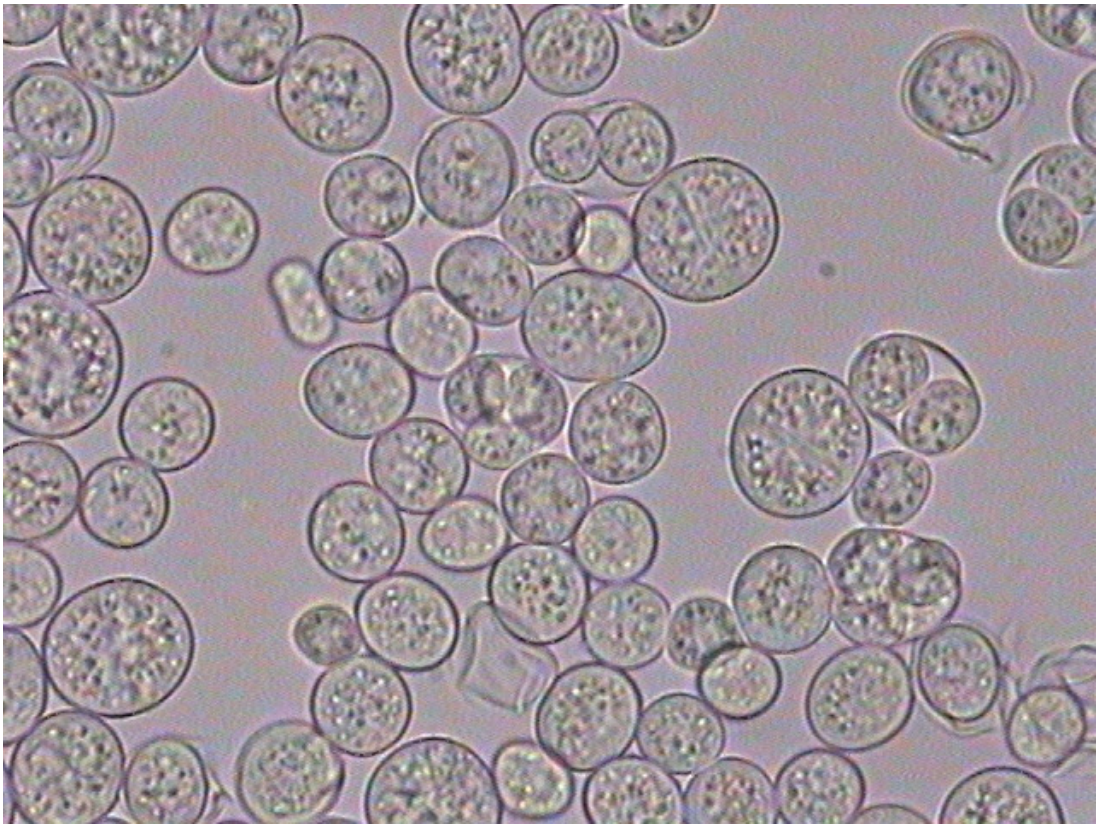


Fig. 17.Sporangia and endospores of *Prototheca zopfii type III,* 48 hours culture, native, wet prep., ×400

6.2.3.2. Observations in cows involved in the farm survey

At the beginning of the *farm survey*, 36 cows were found to excrete *P. zopfii* from a total of 62 quarters. Mixed infection of algae and bacteria (*S. aureus* or *Strep. uberis*) were detected only in 2 cases. The other quarters that were negative for *P. zopfii* (n = 37) were either positive for *S. aureus* and *non agalactiae streptococci (n=18)* or were negative for microorganisms (n=19). No results of microbiological investigations were available before our study.
These 36 *P. zopfii*-infected cows were re-sampled regularly. From the overwhelming majority (n = 34), algae could be isolated continuously during the several months long sampling period. In the *Herd 3* seven cows regularly excreted the pathogen over a 18-month period and the algae survived in the udders also in the pre-calving dry period. After calving, the algae were isolated also from the previously uninfected udder quarters, and in several cases finally all four quarters of the cows became infected with *P. zopfii*. The highest prevalence of this infection was found in *Herd 3*, where 40 alga-excreting cows were identified. During the study period, more than 10% in this herd was continuously infected. In *Herd 3* *P. zopfii* was also cultured from the bulk milk with 2000 colony forming units (CFU) per ml. No mycoplasma was isolated. The faecal samples (from *Herds 2* and *3*), but none of the feed samples yielded *P. zopfii* on culture.

6.2.3.3. Clinical symptoms and pathology

All infected cows (n=36) produced milk with an elevated SCC, which was usually higher than 1 million per ml; however, individual bulk milk values of 6–9 million/ml also occurred. The milk production was very low, only 8–16 (maximum 20) litres.
Systemic clinical symptoms were never seen. 12 of he 62 udder quarters showed slight swelling of the glandular substance that was not or hardly painful on palpation. In chronic cases (n=13) induration and atrophy of the udder quarter were observed. Twelve affected quarters produced milk with a changed gross appearance (barely noticeable decrease in density, presence of small casein clumps or fibrin floccules).
The infected udder quarters of the three cows in which the udder was subjected to gross pathological examination were more compact to the touch than the healthy quarters. The cut surface of the glandular parenchyma was yellowish or rose-red and showed a finely protruding pattern corresponding to the lobules. The milk flowing out onto the cut surface contained a varying number of flakes. The supramammary lymph nodes were enlarged and had a succulent cut surface. Light microscopic histopathological examination showed lesions of different age in the samples taken from the glandular substance of the udder tissue, even within a given udder quarter. In acute cases, the interalveolar connective tissue was widened and infiltrated by lymphocytes, histiocytes and in some foci predominantly by eosinophilic granulocytes. The epithelial cells lining the alveoli appeared to be morphologically intact. The secretion residue seen in the lumen of some alveoli contained neutrophilic granulocytes, many mononuclear cells and *P. zopfii* cells (*Fig. 19*). A large number of PAS-positive alga cells occurred in the interstitium and sometimes such cells were seen also in the glandular epithelial layer (*Fig. 20*). Another characteristic finding was that, parallel with the ageing of lesions, in some glandular lobules the infiltration of the interalveolar interstitium was less pronounced, the secretory epithelial cells of the glandula acini were flattened, and the lumen had dilated and assumed an irregular shape (*Fig. 21*). In some udder quarters circumscribed necrotic foci surrounded by fibroblast proliferation were seen in the area of interstitial inflammation (*Fig. 22*). Occasionally the interlobular and interalveolar connective tissue was markedly widened and only sparsely contained inflammatory cells. Atrophy of the alveoli lined by flattened epithelial cells was observed in the lobules pushed apart by connective tissue (*Fig. 23*). In the supramammary lymph nodes, acute serous lymphadenitis was seen with

marked eosinophilic infiltration in certain foci. The follicles showed signs of expressed hyperplasia.

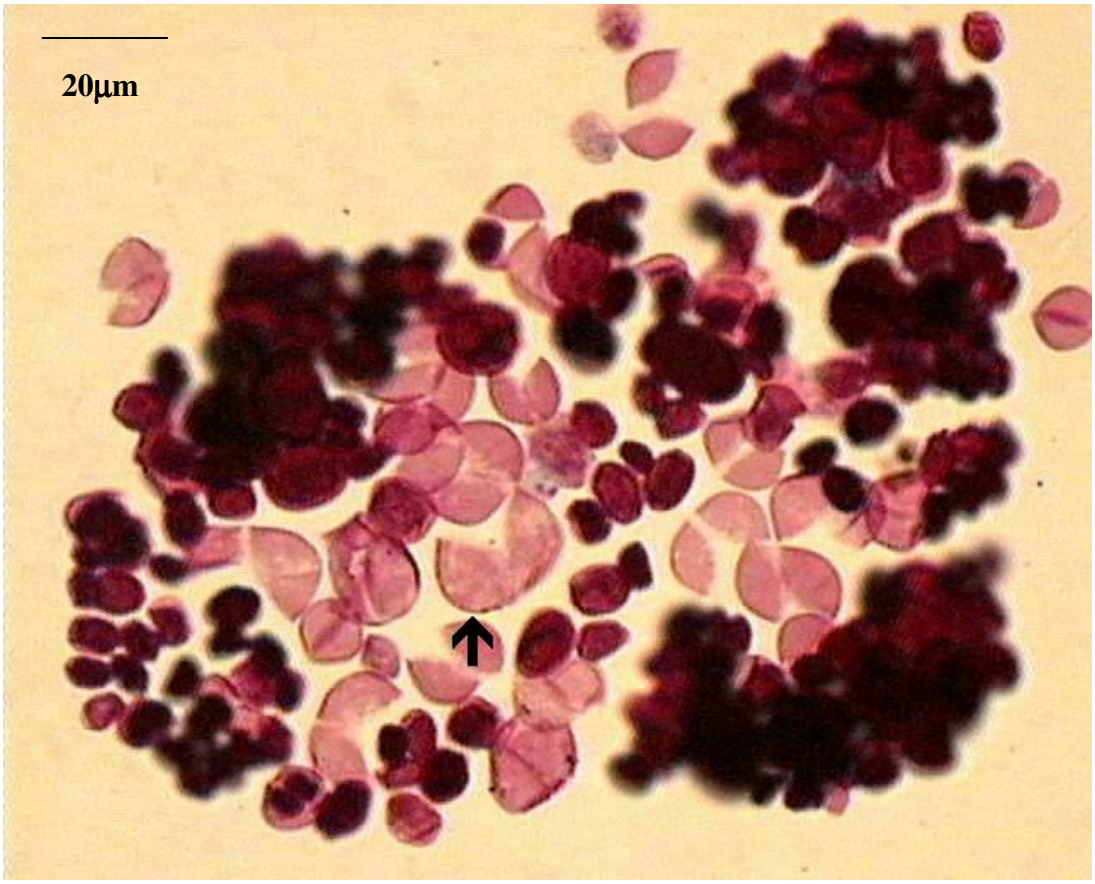


Fig. 18. Cell walls of ruptured sporangia
Periodic acid–Schiff (PAS) reaction, × 400

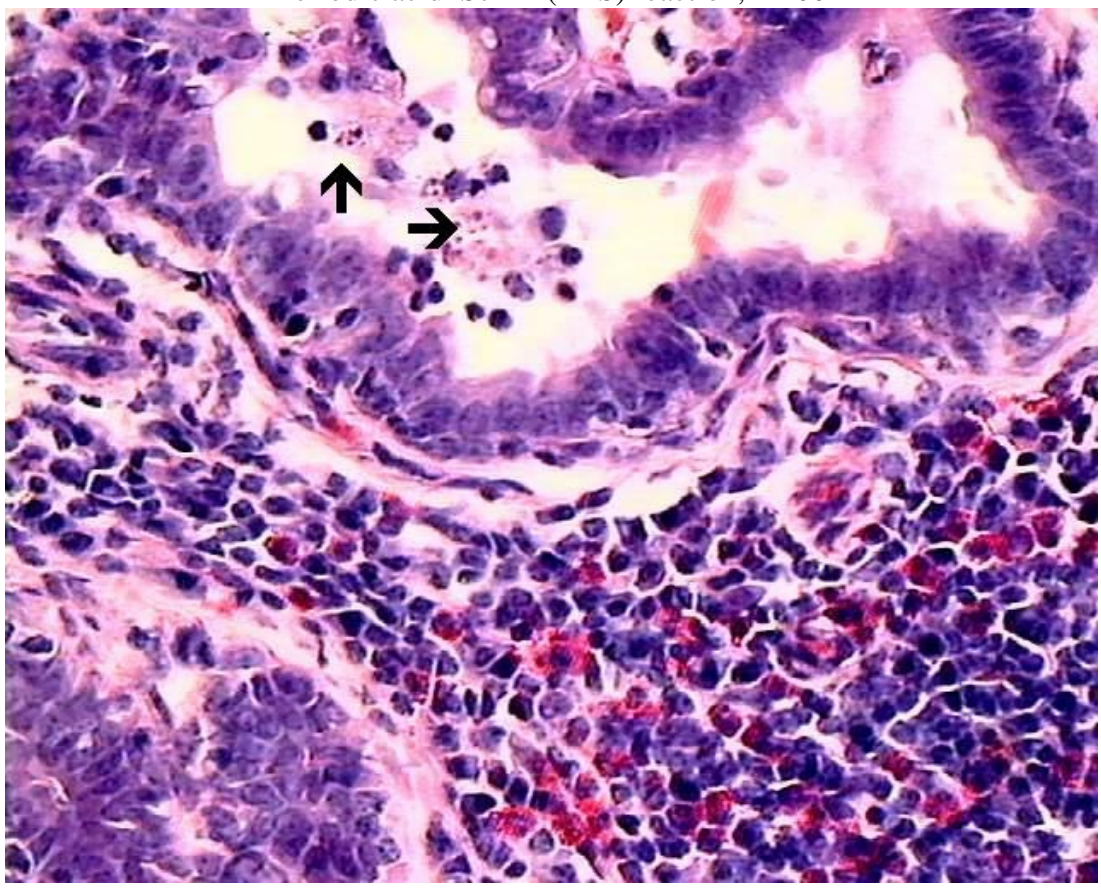


Fig. 19. Acute, productive interstitial inflammation: lympho-histiocytic infiltration with eosinophil cells. Alveoli lined by intact epithelium.Prototheca cells in the lumen (arrows). H.-E. stain, ×400

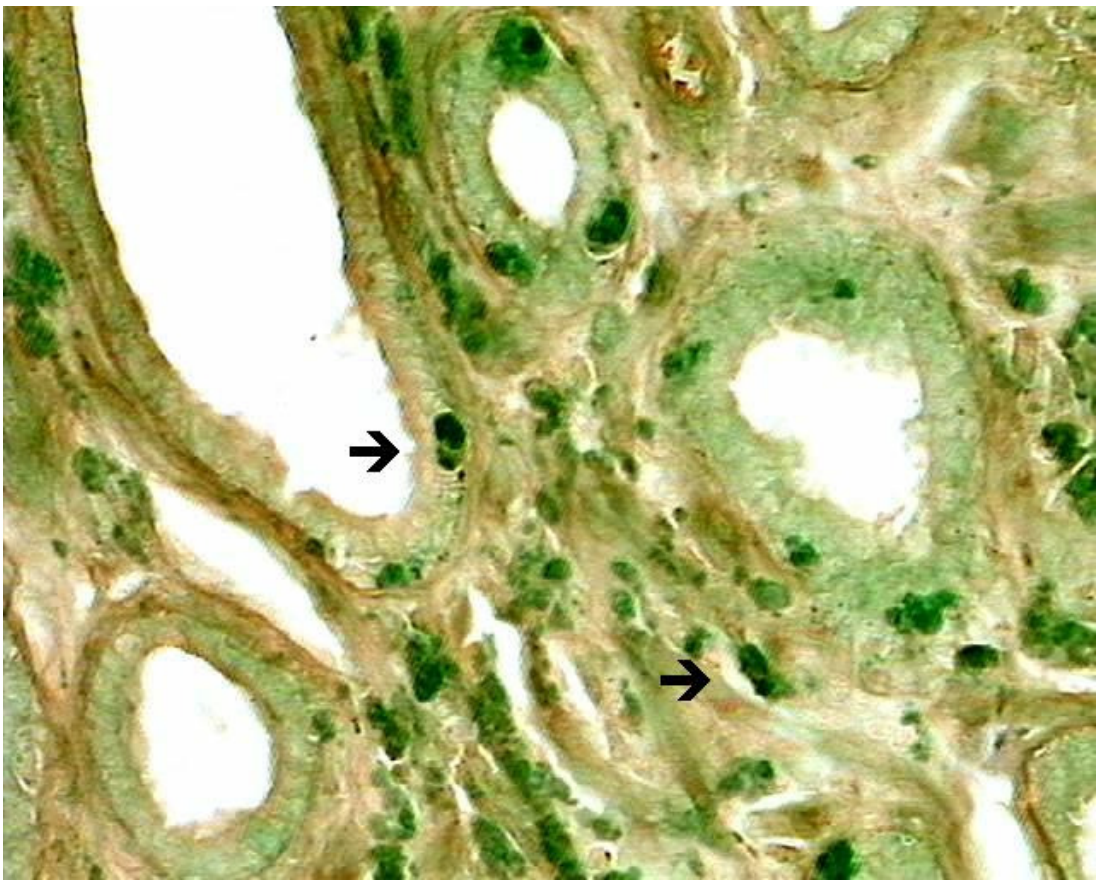


Fig. 20. Prototheca (arrows) in the alveolar epithelial layer and in interstitial spaces. PAS-reaction, (modified colors by computer), ×400

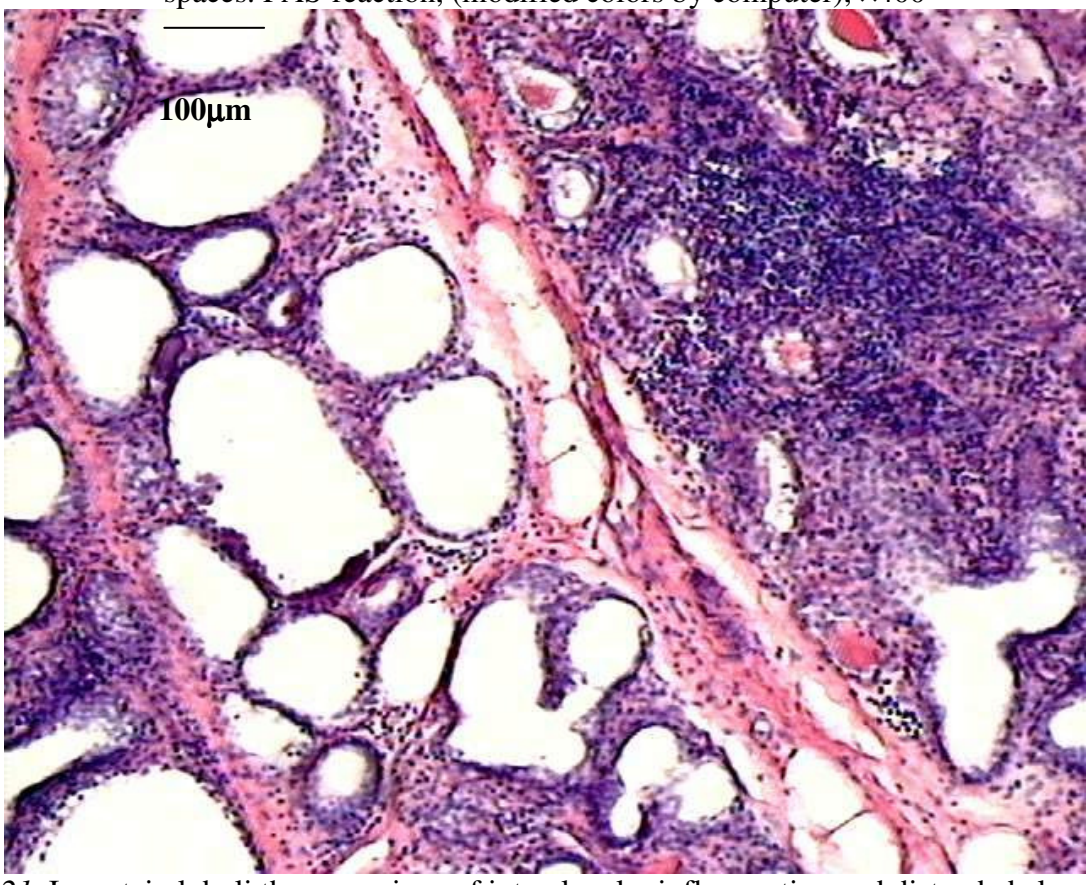


Fig. 21. In certain lobuli there are signs of interalveolar inflammation and distended alveoli lined by a single layer of flattened epithelial cells. H.-E.stain, ×160

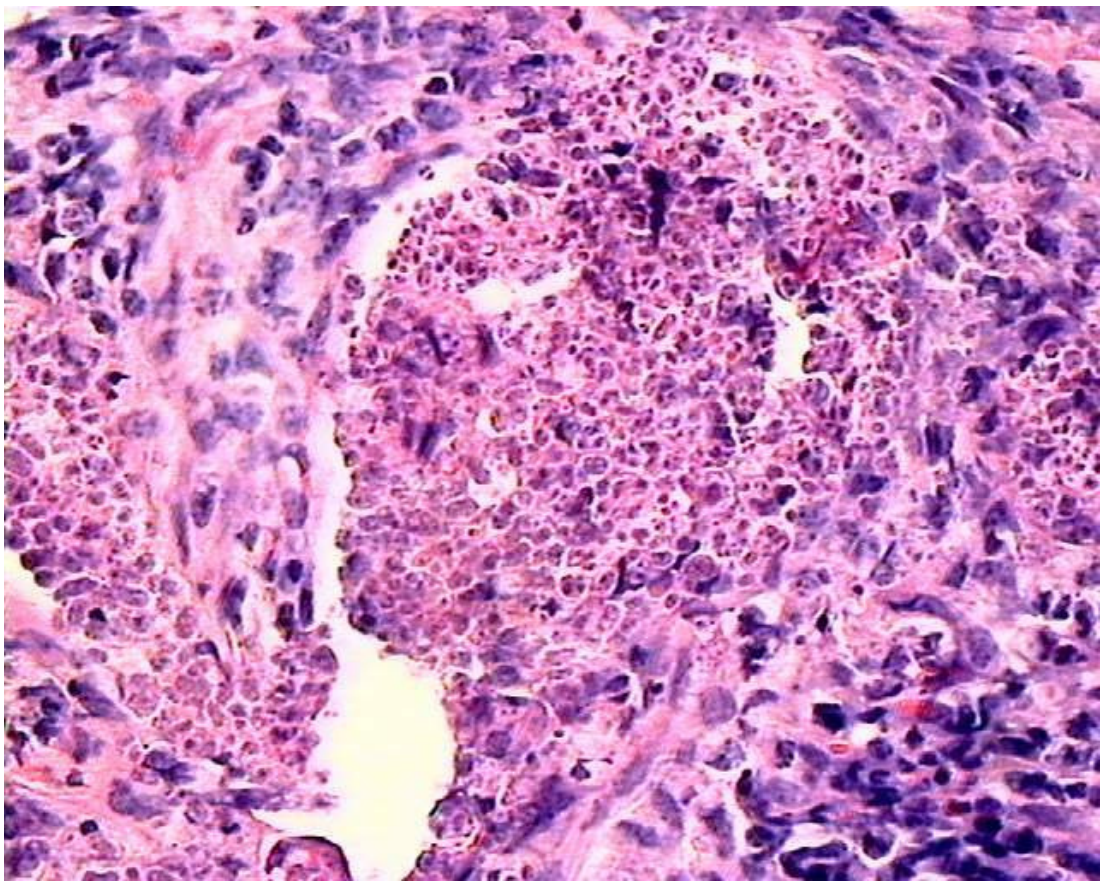


Fig. 22. Necrotic area surrounded by fibroblasts
H.-E.stain, ×400

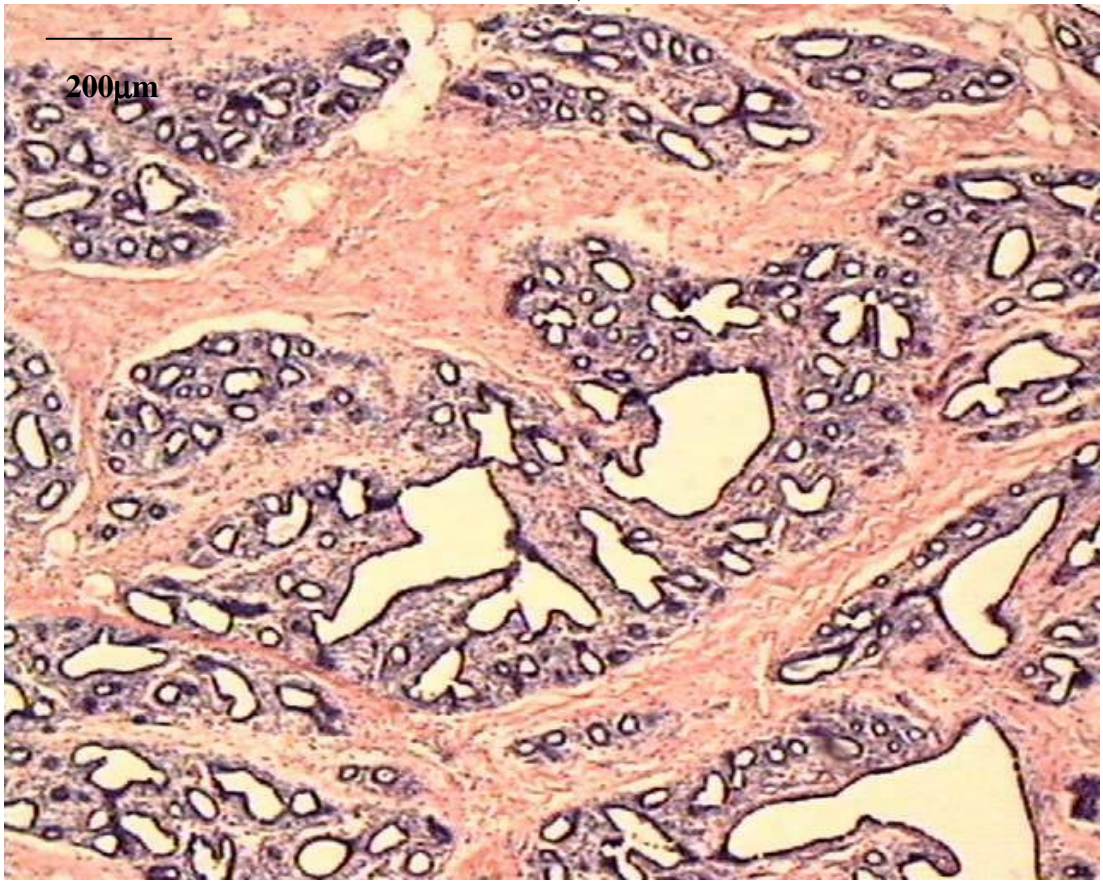


Fig. 23. Alveoli atrophised due to connective tissue proliferation H.-E.stain, ×90

6.2.3.4. Epidemiology

On the basis of their SCC curves over the 18-month study period, the cows found infected in *Herd 3* (n = 40) were classified into three groups.
The cows in *Group 1* (n = 21) produced milk with a high SCC (1–4 million per ml) throughout the whole monitoring period, already at the start of the study. No conclusions on the time and/or source of infection could be made in these cases.
The cows in *Group 2* (n = 9) were in different stages of lactation, and the milk of these cows was characterized by an abrupt rise in the milk SCC in the summer of 1998. (*Fig.24*)

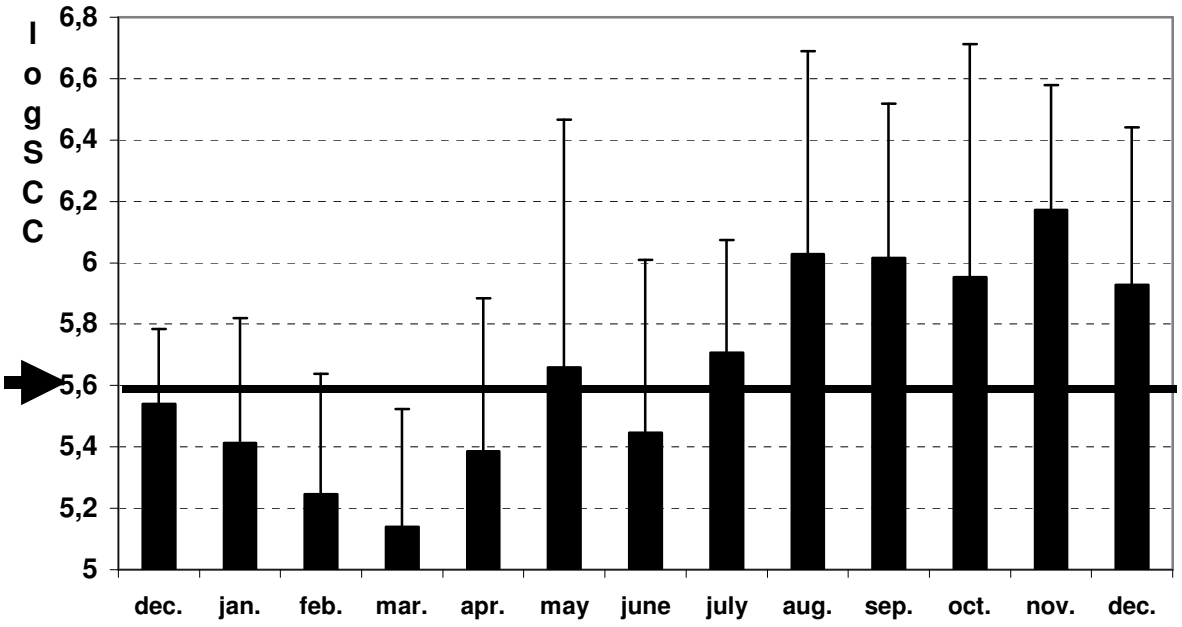


Fig. 24. SCC of cows infected on the summer (n=9); (arrow: 400.000/ml)

The cows in *Group 3* (n = 10) had healthy udders (SCC <400,000 per ml) in their previous lactation. They became infected and the SCC of their milk started to increase immediately after their last calving. Their algal mastitis started to occur independently of seasonal and weather conditions. (*Fig. 25*)

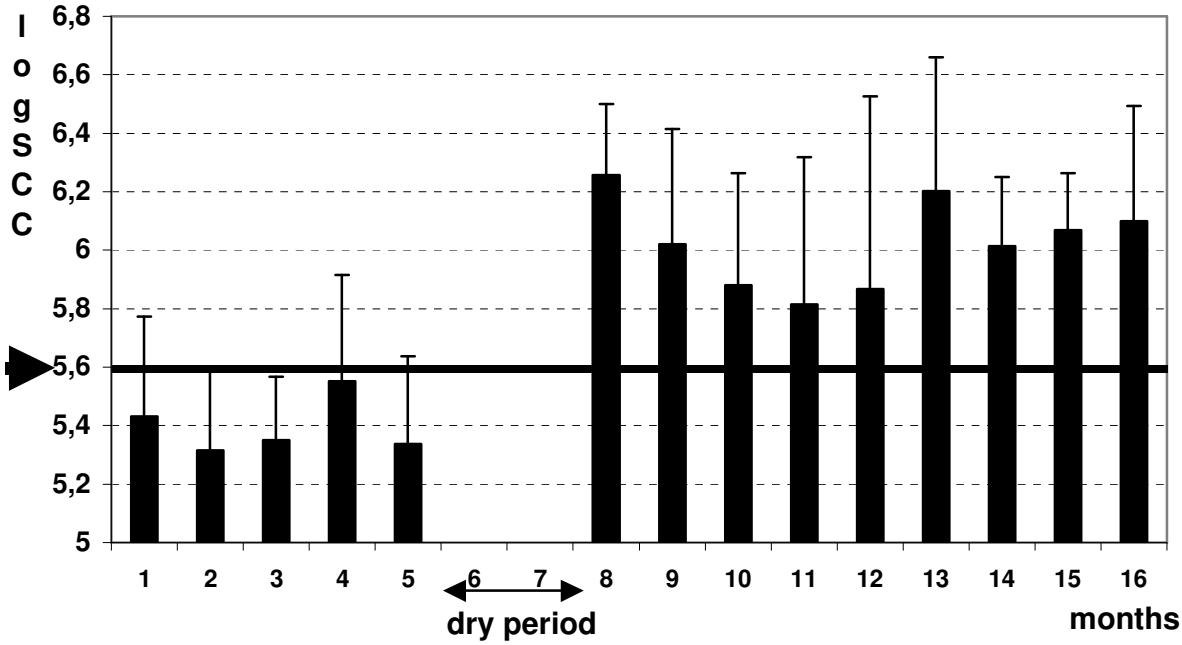


Fig. 25. SCC of cows infected around calving (n=10); (arrow: 400.000/ml)

On these farms, the housing and hygienic conditions and the standard of farm management were below the Hungarian standard (contamination of the milking cups and resting area, animals lying down too early after milking, inexpert intramammary drug administration). When clinical symptoms of mastitis were seen (especially in relapsed cases) several intramammary preparations containing amoxicillin, clavulanic acid, cloxacillin, or the combination of novobiocin, neomycin, penicillin-G, streptomycin and prednisolone were administered. Spontaneous recovery was detected only in three cases soon after first infection. Uncured cows were culled depending on their milk production.

6.2.4. DISCUSSION

These observed cases of algal mastitis were almost always chronic. The most cases occurred in routine diagnostic work were sporadically. The most typical indication for infection was the prolonged SCC elevation. Both the clinical appearance and pathological changes were consistent with those reported earlier, and were typical for *P. zopfii* mastitis (Frank et al., 1969; McDonald et al., 1984b; Schönborn and Seffner, 1977). Almost all of the isolated *P. zopfii* strains belonged to type II, whivh is a recognised causative microbial agent of mastitis (Schuster and Blaschke-Helmessen, 1983; Wilhelm et al., 1992). Three other strains, however, represented the type III of *P. zopfii*. This type has not yet been reported as a mastitis pathogen.

The source of infection could not be determined. Since *P. zopfii* cannot persistently colonize the digestive tract (Pore and Shahan, 1988), the positive faecal samples may suggest the presence of an "active" source of infectious material, such as feed (Baumgartner, 1997). However, we were unable to isolate algae from any of the stored feed. The detection of algae in the faeces and the observed tendency of new infections in group 2 of the epidemiological study support the idea that *P. zopfii* is mainly an environmental mastitis pathogen. These cows must have become infected by heavy exposure to algae that propagated excessively in the animals' environment in the warm weather, first of all in the wet bedding material contaminated with faeces (Baumgärtner, 1997; Costa et al., 1998). Under continental type, temperate zone climatic conditions, the main season for new infections is the summer. The after calving accumulation of new infections in the animals of group 3 suggested that cows might be more sus-

ceptible to *P. zopfii* infection at the start of lactation. Newly lactating cows may have a temporary impairment of intramammary antimicrobial defence mechanisms which predisposes them to environmental mastitis pathogens including *P. zopfii*. The consequences of severe energy imbalance are known factors predisposing the affected individual to clinical mastitis (Suriyasathaporn, 2000), but in the current field study the metabolic status of the cows were not investigated.
Some elements of the technology used on the farms were studied in an attempt to find factors predisposing for a *P. zopfii* infection. Numerous hygienic problems were observed. However, none of them could explain why *P. zopfii* became the predominant mastitis pathogen under these farm conditions (*Herd 3*) instead of bacteria that are known to be environmental pathogens. In *Herd 3* *P. zopfii* mastitis caused enormous financial losses because of the large number of animals infected.

6.2.5. CONCLUSIONS

Based on the results of the farm study and the routine diagnostic work it can be concluded, that *P. zopfii* is a common mastitis pathogen in Hungary. Sporadic algal mastitis cases occur countrywide in large-scale dairy herds. On farms, where there are poor hygienic conditions and faulty management system even endemic forms of *P. zopfii* mastitis may occur. In most cases, *P. zopfii* infection may lead to mild, but chronic inflammatory process in the udder, resulting in fibrosis and alveolar atrophy with dramatic loss of milk production and permanent increase in SCC.

From most algal mastitis cases type II of P. zopfii was isolated, but from 3 cases type III was detected, which has not been reported as mastitis pathogen previously.

7. OVERVIEW OF THE NEW SCIENTIFIC RESULTS

1.) We found a significant mastitis-predictive value of the elevated BHB level postpartum, but not to any other of NEB related changes in circulating levels of hormones and metabolites. This predictive value was highly significant for GN microbes. Based on these findings we suppose that hyperketonaemia, rather than NEB by itself can predispose cows for mastitis in the early weeks of lactation. The data of cows with *S. aureus* IMI were very close to their healthy herdmates.

2.) We found elevated BHB levels in cows with NDP+GN mastitis within some hours after the outbreak of clinical symptoms. This BHB dependent character of NDP+GN mastitis was obvious in the first four weeks after calving. We observed a temporary NEFA increase in cows with NDP+GN mastitis, which might be the catabolic consequence of endotoxin induced endocrine changes.
We observed a significant decrease in plasma levels of both T_4 and T_3, an increase of rT_3 level, and a diminished TRH-challenged T_4 and T_3 response in NDP+GN mastitis. These mastitis related changes were dramatic in the few cows died of mastitis soon after sampling. This observations reveal that the mastitis related endotoxin loading in cows may decrease the 5'D-dependent activation of T_4 to T_3 and increase the capacity of its 5D-catalyzed inactivation to rT_3.

3.) We observed a temporary elevation in plasma insulin level in the first 2-3 samples of our cases with NDP+GN mastitis. In complete agreement with the findings of model studies the IGF-I level was still almost unaffected at the time of this insulin elevation, and started to decrease continuously thereafter. These results confirmed that the endotoxin-induced changes of both GH-IGF-I axis and insulin participate in the shift of the metabolism towards catabolic events in ruminants including the postpartum dairy cows as well.

4.) About 23 % of the examined cows were considered as *temporary hypocorticoid*. Neither the baseline level of cortisol, nor the degree of ACTH-induced cortisol response predisposed cows for mastitis. However,in cows with NDP+GN mastitis in the early puerperal phase the ACTH challenged cortisol increment inversely related to the severity of clinical symptoms. These experiences confirmed the importance of *temporary hypocorticism* and the regulatory role of physiological cortisol response in production and release of certain interleukins and TNFα in GN mastitis.

5.) We found a similarly low recovery rate of bacteria after a single-dose IM treatment as seen in the untreated controls. After the *intracisternal* and *4 IM* groups the bacteriological recovery rates were significantly (*4 IM* group) higher but remained below 50%. Based upon these results we did not give extra preference to the systemic application of spiramycin at drying off in the practice comparing to treatments with other commercially available drugs.

6.) We isolated *Prototheca zopfii* alga from several hundreds of mastitic cows from more than 50 dairy herds. This form of mastitis had not been reported in Hungary previously. We found an increasing tendency in the number of identified cases in the last years.The *type III* variant of *P. zopfii* isolated from 3 cows had not been reported as a mastitis pathogen. The early weeks of lactation and the summer wheather predisposed the cows to the new algal IMI. The *P. zopfii* infection usually resulted in a chronic subclinical, or a mild clinical, inflammatory process in the udder followed by a dramatic loss in milk production and permanent increase in somatic cell count. Prototheca zopfii is concluded as a common mastitis pathogen of dairy cows in Hungary

8. REFERENCES

Aalbaek B, Jensen HE, and Huda A. Identification of *Prototheca* from bovine mastitis in Denmark. Acta Pathol Microbiol Immunol Scand 1988; 106: 483–488.

Aiumlamai S. Studies on effects of inhibition of prostaglandin biosynthesis, and on prostaglandins and endotoxin-related diseases in ruminants. PhD. Dissertation. Faculty of Veterinary Medicine, Uppsala, 1991

Akers RM, and Nickerson SC. Effects of prepartum blockade of microtubule formation on milk production and biochemical differentiation of the mammary epithelium in Holstein heifers. Int J Biochem 1983; 15: 777-788.

Alan MGS, Dobson H, and Fitzpatrick RJ. Endocrine response to different doses of ACTH in cows. British Vet J 1986; 142, 239-245.

Ali-Vehmas T, Vikerpuur M, Fang W, and Sandholm M. Giving selenium supplements to dairy cows strengthens the inflammatory response to intramammary infection, and induces a growth-suppressing effect on mastitis pathogens in whey. J Vet Med A 1997; 44: 559-571.

Andersen PH, Bergelin B, and Christensen KA. Effect of feeding regimen on concentration of free endotoxin in ruminal fluid in cattle. J Anim Sci 1994a; 72: 487-491.

Andersen PH, Hesselholt M, and Jarlov N. Endotoxin and arachidonic acid metabolites in portal, hepatic and arterial blood of cattle with acute ruminal acidosis. Acta Vet Scand 1994b; 35: 223-234.

Andersen PH, Jarlov N, and Hesselholt M. Investigation of possible role of endotoxin, TXA_2, PGI_2 and PGE_2 in experimentally induced rumen acidosis in cattle. Acta Vet Scand 1990; 31: 27-38.

Andersen PH,. Jarlov N, Hesselholt M, and Bek L. Studies on in vivo endotoxin plasma disappearance times in cattle. J Vet Med A 1996; 43: 93-101.

Anderson KL, and Walker RL. Sources of *Prototheca* spp. in a dairy herd environment. J. Am Vet Med Assoc 1988; 193: 553–556.

Anderson KL, Kindahl H, Smith AR, Davis LE, and Gustafsson K. Endotoxin -induced bovine mastitis: arachidonic acid metabolites in milk and plasma and effect of flunixin meglumine. Am J Vet Res 1986; 47: 1373-1377.

Awadeh FT, Kincaid RL,. and Johnson KA. Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. J Anim Sci 1998; 76: 1204-1215.

Bage R, Forsberg M, Gustafsson H, Larsson B, and Rodriguez-Martinez H.. Effect of ACTH-challenged on progesterone and cortisol levels in ovariectomized repeat breeder heifers. Anim Reprod Sci 2000; 63: 65-76.

Barber AE, Coyle SM, Marano MA, Fischer E, Calvano SE, Fong Y, Moldawer LL, and Lowry SF. Glucocorticoid therapy alters hormonal and cytokine responses to endotoxin in man. J Immunol 1993; 150: 1999-2006.

Barber MR, and Yang TJ. Chemotactic activities in nonmastitic, and mastitic mamary secretions: presence of interleukin-8 in mastitic but not nonmastitic secretions. Clin Diagn Lab Immunol 1998; 5: 82-86.

Barkema HW, Schukken YH, Lam TJ, Beiboer ML, Wilmink H, Benedictus G, and Brand A. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J Dairy Sci 1998; 81: 411-419.

Bartalena LF, Bogazzi F, Brogioni S, Grasso L, and Martino E. Role of cytokines in the pathogenesis of the euthyroid sick syndrome. Eur J Endocrinol 1998; 138: 603-614.

Batra TR, Hidiroglou M, and Smith MW. Effect of vitamin E on incidence of mastits in dairy cattle. Can J An Sci 1992; 72: 287-297.

Battaglia DF, Beaver AB, Harris TG, Tanhehco E, Viguie C, and Karsch FJ. Endotoxin disrupts the estradiol-induced luteinizing hormone surge: interference with estradiol signal reading, not surge release. Endocr Philadelph 1999; 140: 2471-2479.

Battaglia DF, Krasa HB, Padmanabhan V, Viguie C, Karsch FJ, and Vasantha-Padmanabhan. Endocrine alterations that underlie endotoxin.induced disruption of the follicular phase in ewes. Biol Reprod 2000; 62: 45-53.

Baumgärtner B. Occurrence and control of bovine mastitis caused by Prototheca in the catchment area covered by the Potsdam State Institute for Veterinary and Food Analysis. (in German) Der praktische Tierarzt 1997; 78: 406–414.

Beaudeau F, Fourichon C, Seegers H, and Bareille N. Risk of clinical mastitis in dairy herds with a high proportion of low individual milk somatic cell count. Prev Vet Med 2002; 53: 43-54.

Berczi I.. Neuroendocrine defense in endotoxin shock. Acta Microbiol Hung 1993; 40: 265-302.

Bergmann A. Experimental Prototheca mastitis in the cow and its treatment with tetramisole hydrochloride. (in German) Berl Münch Tierärztl Wschr 1993; 106: 257–260.

Bernal A, DeMoraes GV, Thrift Willard CC, and Randel RD. Effects of induced hypothyroidism on ovarian response to superovulation in Brahman (Bos indicus) cows. J Anim Sci 1999; 77: 2749-2756.

Bertók L, and U. Nagy Zs. The effect of endotoxin and radio-detoxified endotoxin on the serum T_4 level of rats and response of their thyroid gland to exogenous TSH. Immunopharmacology 1984; 8: 143-146.

Bertók L. Bacterial endotoxins and nonspecific resistance. In: Ninnemann, J.L. (ed): Traumatic Injury: Infection and other immunologic saequelae University Park Press, Baltimore, MD. 1983; p.119-143.

Bertók L. Endotoxins and endocrine system. Dom Anim Endocrin 1998; 15: 305-308.

Blaschke-Hellmessen R, Schuster H. Bergmann V. Differentiation of the variants of *Prototheca zopfii* Krüger 1894. (in German) Arch exper Vet Med 1985; 39: 387-397.

Blaschke-Hellmessen R, Schuster, H. Survival of *Prototheca zopfii* and *Prototheca wickerhamii* under various environmental conditions. (in German) Mh Vet Med 1984; 39: 852-856.

Blogg JR, and Sykes JE. Sudden blindness associated with protothecosis in a dog. Austr Vet J 1995; 72: 147–149.

Blum JW, Bruckmaier RM, Vacher PY, Münger A,. and Jans F. Twenty-four-hour patterns of hormones and metabolites in week 9 and 19 of lactation in high-yielding dairy cows fed tryglycerides and free fatty acids. J Vet Med A 2000; 47: 43-60.

Boddie RL, and Nickerson SC. Dry cow therapy: effects of method of drug administration occurrence of intramammary infection. J Dairy Sci. 1986; 69: 253-257.

Bolourchi M, Hovareshti P, and Tabatayi AH. Comparison of the effects of local and systemic dry cow therapy for staphylococcal mastitis control. Prev Vet Med 1996; 25: 63-67.

Bosu WTK, and Peter AT. Evidence for a role of intrauterine injections in the pathogenesis of cystic ovaries in dairy cows. Theriogenology 1987; 28: 725-736.

Bosu WTK, Kujjo LLL, and Perez GI. Endotoxin stimulates secretion of cortisol but not ACTH in heifers pretreated with suppressive doses of a glucocorticoid. J Vet Med A 1995; 42: 1-8.

Boudjellab N, Chang-Tang HS, Li X, and Zhao X. Interleukin-8 response by bovine mammary epithelial cells to lipopolysaccharide stimulation. Am J Vet Res 1998; 59: 1563-1567.

Boyd TM, Oliver SP, and Maki JL. Transfer of antibiotics from treated to untreated mammary quarters during the first week of the dry period. J Dairy Sci 1987; 70(Suppl. 1): 247 (Abstr.)

Bramley AJ, and Dodd FH. Reviews of the progress of dairy science: mastitis control-progress and prospects. J Dairy Res 1984; 51:481-512.

Briard N, Dadoun F, Pommier G, Sauze N, Lebouc Y, Oliver C, and Dutour A. IGF-I /IGFBPs system response to endotoxin challenge in sheep. J Endocr 2000; 164: 361-369.

Browning JW, Mein GA, Barton M, Nicholls TJ, and Brightling P. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. Austral Vet J 1990; 67: 440-442.

Browning JW, Mein GA, Brightling P, Nicholls TJ, and Barton M. Strategies for mastitis control: dry cow therapy and culling. Austral Vet J 1994; 71: 179-181.

Browning R, Gissendanner SJ, and Wakefield T. Ergotamine alters plasma concentrations of glucagon, insulin, cortisol, and triiodothyronine in cows. J Anim Sci 2000; 78: 690-698.

Browning R, Leite-Browning ML, Smith HM, and Wakefield T. Effect of ergotamine and ergonovine on plasma concentrations of thyroid hormones and cortisol in cattle. J Anim Sci 1998; 76:1644-1650.

Bruss ML. Lipids and ketones. In. Kaneko,. JJ, Harvey,. JW, Bruss, M L (eds). Clinical biochemistry of domestic animals Academic Press, San Diego, London, Boston, New York, Sydney, Tokio, Toronto, 1997; 83-115.

Bunting LD, Fernandez. JM, Fornea RJ, White TW, Froetschel MA, Stone. JD, and Ingawa K. Seasonal effects of supplemental fat or undegradable protein on the growth and metabolism of Holstein calves. J Dairy Sci 1996; 79: 1611-1620

Cai TQ, Weston PG, Lund LA, Brodie B, McKenna DJ, and Wagner WC. Association between neutrophil functions, and periparturient disorders in cows. Am J Vet Res 1994; 55: 934-943.

Capuco AV, Keys. JE, and Smith. JJ. Somatotrophin increases thyroxine-5-monoiodinase activity in lactating mammary tissue of the cow. J Endocrinol 1989; 121: 205-211.

Capuco AV, Wood DL, Elsasser TH, Kahl S, Erdman RA, Van Tassell CP, Lefcourt A, and Piperova LS. Effect of somatotropin on thyroid hormones and cytokines in lactating dairy cows during ad libitum and restricted feed intake. J Dairy Sci 2001; 84: 2430-2439.

Carey WP, Kaykova Y, Bandres JC, Sidhu GS, and Brau N. Cutaneous protothecosis in a patient with AIDS and a severe functional neutrophil defect: successful therapy with amphotericin B Clin Infect Dis 1997; 25: 1265–1266.

Casal M, and Gutierrez J. Simple new test for presumptive differentiation between genus *Candida* and genus *Prototheca*. Mycopathol 1986; 94: 3–5.

Casal MJ, and Aroca JG. Simple new test for rapid differentiation of *Prototheca stagnora* from *P. wickerhamii* and *P. zopfii*. Mycopathol 1995; 130: 93–94.

Cassar-Malek I, Kahl S,. Jurie C, and Picard C. Influence of feeding level during postweaning growth on circulating concentrations of thyroid hormones and extrathyroidal 5'-deiodination in steers. J Anim Sci 2001; 79: 2679-2687.

Cheville NF, McDonald J, and Richard J. Ultrastructure of *Prototheca zopfii* in bovine granulomatous mastitis. Vet Pathol 1984; 21: 341–348.

Chew BP. Role of carotenoids in the immune response. J Dairy Sci 1993; 76: 2804-2811.

Chew BP. Vitamin A, and β-carotene on host defense. J Dairy Sci 1987; 70: 2732-2743.

Clemmons DR, and Underwood LE. Role of insulin-like growth factors and growth hormone in reversing catabolic states. Hormone Research 1992; 38: 37-40.

Coppock CE, Everett RW, Natzke RP, Ainslie HR. Effect of dry period length on Holstein milk production and selected disorders at parturition. J Dairy Sci 1974; 57: 712-718.

Correa MT, Erb H, and Scarlett J. Path analysis for seven postpartum disorders of Holstein cows. J Dairy Sci 1993; 76: 1305-1312.

Cort N, and Kindahl H. Endotoxin induced abortion in early pregnant gilts and its prevention by flunixin meglumine. Acta Vet Scand 1990; 31: 347-358.

Cort N, and Kindahl H. The effect of a bacterial endotoxin or cloprostenol on the clinical status and hormonal levels in 80-100 days pregnant gilts. Acta Vet Scand 1986; 27: 145-158.

Cort N, Fredriksson G, Kindahl H, Edqvist LE, and Rylander R. A clinical and endocrine study on the effect of orally administered bacterial endotoxin in adult pigs and goats. J Vet Med A 1990; 37: 130-137.

Costa EO, Carciofi AC, Melville PA, Prada MS, and Schalch U. *Prototheca* sp. outbreak of bovine mastitis. J Vet Med 1996a; 43: 321–324.

Costa EO, Melville PA, Ribeiro AR, Watanabe ET, and Parolari MCFF. Epidemiologic study of environmental sources in a *Prototheca zopfii* outbreak of bovine mastitis. Mycopathol 1997; 137: 33–36.

Costa EO, Ribeiro AR, Melville PA, Prada MS, Carciofi AC, and Watanabe ET. Bovine mastitis due to algae of the genus *Prototheca*. Mycopathol 1996b; 133: 2, 85-88.

Costa EO, Ribeiro AR, Watanabe ET, Pardo RB, Silva JAB, and Sanches RB. An increased incidence of mastitis caused by *Prototheca* species and *Nocardia* species on a farm in Sao Paulo, Brazil. Vet Res Commun 1996c; 20: 237–241.

Costa EO, Ribeiro AR., Watanabe ET, and Melville PA. Infectious bovine mastitis caused by environmental organisms. J Vet Med B 1998; 45: 2, 65-71.

Craven N. Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation – a review. Br Vet J 1987; 143: 410-422.

Cullor JS. Shock attributable to bacteremia and endotoxemia in cattle: Clinical and experimental findings. J Am Vet Res 1992; 200: 1894-1902.

Csernus V. Antibodies of high affinity and specificity for RIA determination of progesterone, testosterone, estradiol-17β and cortisol. (in Hungarian) In. Görög, S (ed). Advances in steroid analysis I Acad Press, Budapest, Hungary 1982.

Daels PF, Stabenfeldt GH, Hughes JP, Odensvik K, and Kindahl H. Effects of flunixin meglumine on endotoxin-induced $PGF_{2\alpha}$ secretion during early pregnancy in mares. Amer J Vet Res 1991a; 52: 276-281.

Daels PF, Stabenfeldt GH, Hughes JP, Odensvik K, and Kindahl H. Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. Am J Vet Res 1991b; 52: 282-288.

Daels PF, Starr M, Kindahl H, Fredriksson G, Hughes JP, and Stabenfeldt GH. Effect of Salmonella typhimurium endotoxin on $PGF_{2\alpha}$ release and fetal death in the mare. J Reprod Fert (Suppl.) 1987; 35: 485-492.

Deitch EA, Winterton J, Li M, and Berg R. The gut as a portal entry for bacteremia. Ann Surg 1987; 205: 681-692.

Desnottes J F, and Diallo N. Comparative study of intramacrophagic penetration and action on phagocytosis of a macrolide (spiramycin) and a fluoroquinolone (pefloxacin). Pathol Biol Paris 1990; 38: 281-285.

Desnottes JF, Diallo N, and Moret G. Effect of spiramycin on adhesiveness and phagocytosis of gram.positive cocci. J Antimicrob Chemother 1988; 22: 25-32.

Destilleux JC, Gröhn YT, and Quaas RL. Effects of clinical ketosis on test day milk yields in Finnish Ayrhire cattle. J Dairy Sci 1994; 77: 3316-3323.

Dickson WM. Endocrine glands In. Swenson MJ (ed). Dukes' Physiology of Domestic Animals. Tenth edition Cornell University Press, Ithaca, 1990; pp 761-797.

Dillberger JE, Homer B, Daubert D, and Altman NH. Protothecosis in two cats. J Am Vet Med Assoc 1988; 192: 1557–1559.

Dinarello CA. Molecular mechanisms in endotoxin fever. Agents Actions 1983; 13: 470-474.

Dion WM. Bovine mastitis due to *Prototheca zopfii* II. Can Vet J 1982; 23: 272–275.

Dodd FH. Mastitis-progress on control. J Dairy Sci 1983; 66: 1773-1780.

Dodge JD. The fine structure of algal cells. Academic Press, London-New York. 1973; p. 261.

Dosogne H, Burvenich C, Van Weven T, Roets E, Nordhuizen-Stassen EN, and Godderis B. Increased surface expression of CD11b receptors on polymorphonuclear leukocytes is not sufficient to sustain phagocytosis during Escherichia coli mastitis in early postpartum dairy cows. Vet Immunol Immunopathol 1997; 60: 47-59.

Douglas RG, Gluckman PD, Breier BH, McCall JL, Parry B, and Shaw JHF. Effects of recombinant IGF-1 on protein and glucose metabolism in rTHF-infused lambs. Am J Physiol 1991; 261: 606-612.

Dutt K. The growth of some common mammary pathogens in the secretions of the bovine mammary gland during the dry period. M.S. Thesis, Pennsylvania State Univ., University Park; 1985.

Eberhart RJ, and Buckalew JM. Evaluation of a hygiene and dry period therapy program for mastitis control. J Dairy Sci 1972; 55: 1683-1691.

Eberhart RJ, and Buckalew JM. Intramammary infections in a dairy herd with low incidence of Streptococcus agalactiae and Staphylococcus aureus infections. J Am Vet Med Assoc 1977; 171: 630-634.

Eberhart RJ. Management of dry cows to reduce mastitis. J Dairy Sci 1986; 69: 1721-1732.

Elenkov IJ, Kiss E, Stark E, and Bertók L. CRF-dependent and CRF-independent mechanisms involved in hypophyseal adrenal system activation by bacterial endotoxin. Acta Physiol Hung 1992; 79: 355-363.

Elsasser TH, Caperna T.J, and Rumsey TS. Endotoxin administration decreases plasma insulin-like growth factor (IGF)-1 and IGF-binding protein-2 in Angus x Hereford steers independent of changes in nutritional intake. J Endocrinol 1995; 144: 109-117.

Elsasser TH, Fayer R, Rumsey TS, and Hartnell GF. Recombinant bovine somatotropin blunts plasma tumor necrosis factor-α, cortisol and thromboxane-B_2 responses to endotoxin in vivo. Endocrinology 1994; 134: 1082-1088.

Elsasser TH, Richards M, Collier R, and Hartnell GF. Physiological responses to repeat endotoxin challenge are selectively affected by recombinant bovine somatotropin administration to calves. Domestic Animal Endocrinol 1996; 13: 91-103.

Elsasser TH, Rumsey TS, and Hammond AC. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-1 in beef cattle. J Anim Sci 1989; 67: 128-137.

Elsasser TH, Rumsey TS, Hammond AC, and Fayer R. Influence of parasitism in plasma concentrations of growth hormone, somatomedin-C and somatomedin-binding proteins in calves. J Endocrinology 1988; 116: 191-200.

Erb HN, and Gröhn YT. Epidemiology of metabolic disorders in the periparturient dairy cow. J Dairy Sci 1988; 71: 2557-2571.

Erskine RJ, Bartlett PC, Crawshaw PC, and Gombas DM. Efficacy of intramuscular oxytetracycline as a dry cow treatment for Staphylococcus aureus mastitis. J Dairy Sci 1994; 77: 3347-3353.

Filar J, KandeferSzerszen M, Szuster-Ciesielska A and Rzeski W. Effects of cold treatment and ketosis induced by starvation on interferon production in leukocytes of lactating cows. (in German) Dtsch Tieraerztl Wochenschr 1992; 99: 210-213.

Fliesen T, Maiter D, Gerard G, Underwood LE, Maes M, and Ketelslegers JM. Reduction of serum insulin-like growth factor-I by dietary protein restriction is age dependent. Pediatric Res 1989; 26: 415-419.

Forbes GB, Brown MR, Welle SL, and Underwood LE. Hormonal response to overfeeding. Amer Can J Clin Nutr 1989; 49: 608-611.

Forshell KP, Osteras O, Aagaard K, and Kulkas L. Antimicrobial drug policy in four Nordic countries. Mastitis Newsletter 1996; 21: 26-28.

Fox ES, Thomas P, and Broitman SA. Hepatic mechanisms for clearance and detoxification of bacterial endotoxins. J Nutr Biochem 1990; 1: 620-628.

Frank N, Ferguson LC, Cross RF, and Redman DR. *Prototheca*, a cause of bovine mastitis. Am J Vet Res 1969; 30: 1785–1794.

Franklin A, Horn af Raintzen M, Obel N, Östensson K, Aström G. Concentrations of penicillin, streptomycin, and spiramycin in bovine udder tissue liquids. Am J Vet Res 1986; 47: 804-807.

Franklin ST, Young JW, and Nonnecke BJ. Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. J Dairy Sci 1991; 74: 2507-2514.

Fredriksson G, Kindahl H, and Edqvist LE. Endotoxin-induced prostaglandin release and corpus luteum function in goats. Anim Reprod Sci 1985; 8: 109-121.

Furuoka H, Anri A, Arita Y, Tuzuki N, Satoh, and Itakura C. *Protothecal* mastitis in a cow. Jap J Vet Sci 1989; 51: 1, 197-199.

Gacs M, Huszenicza Gy, Kégl T, Vígh Zs, Stollár Zs, Oláh B,. Johnsson, P, and Oppel K. Experiences on the effectiveness of an intramammary infusion without containing antibiotics I Incidence and clinical features of mastitis with different bacteriological backgrounds on seven large-scale dairy farms (In Hungarian). Magy Áo Lapja, 1993; 48: 473-497.

Garcia-Villar R, Marnet PG, Laurantie MP, and Toutain PL. Relative oxytocic properties of fenprostalene compared with cloprostenol, prostaglandin $F_{2\alpha}$ and oxytocin in the ovariectomized ewe. Am J Vet Res 1985; 46: 841-844.

Gedek B, Weber A.: *Prototheca* mastitis of the cows is a herdproblem. (in German)Tierärztl Prax 1978; 6: 441-450.

Gennano-Soffietti M, Nebbia C, Biolatti B, Re G, Castagnaro M, Cottino F, and Guarda F. Toxicology of fungicides. effects of 270 days administration of zinc-ethylene-bis-dithiocarbamate in Friesian cattle. (in German) Schweiz Arch Tierheilk 1988; 130: 657-672.

Gilbert RO, Bosu WTK, and Peter AT. The effect of Escherichia coli endotoxin on luteal function in Holstein heifers. Theriogenology 1990; 33: 645-651.

Ginel PJ, Perez J, Molleda JM, Lucena R, and Mozos E. Cutaneous protothecosis in a dog. Vet Rec 1997; 140: 651–653.

Giri SN, Chen Z, and Carrol EJ. Role of prostaglandins in pathogenesis of bovine mastitis induced by Escherichia coli endotoxin. Am J Vet Res 1984; 45: 586-591.

Giri SN, Emau P, Cullor JS, Stabenfeldt GH, Bruss ML, Bondurant RH, and Osburn BL. Effects of endotoxin infusion on circulating levels of eicosanoids, progesterone, cortisol, glucose and lactic acid and abortion in pregnant cows. Vet Microbiol 1990; 21: 211-231.

Goudswaard J. Protothecosis in a cow. (in Dutch) Tijdschr Dierg 1977, 102: 567-570.

Green MJ, Green LE, and Cripps PJ. Low bulk milk somatic cell counts and endotoxin-associated (toxic) mastitis. Vet Rec 1996; 138: 305-306.

Gröhn YT, Erb HN, McCulloch CE, and Saloniemi H. Epidemiology of metabolic disorders in dairy cattle and association among host characteristics, disease, and production. J Dairy Sci 1989; 72: 1876-1885.

Gröhn, Y.T., Hertl, J.A. and Harman, J.L. Effect of early lactation milk yield on reproductive disorders in dairy cows. Am J Vet Res 1994; 55: 1521-1528.

Haastaren vaan GAC, Meer van der MJM, Hermus ARMM, Linkels E, Klootwijk W, Kaptein E, Toor van H, Sweep CGJ, Visser TJ, and Greef de WJ. Different effects of continuous infusion of IL-1 and IL-6 on the hypothalamic-hypophyseal-thyroid axis. Endocrinol 1994; 135: 1336-1345.

Hamir AN, and Parry OB. An abattoir study of bovine neoplasms with particular reference to ocular squamous cell carcinoma in Canada. Vet Rec 1980; 106: 551-553.

Haraszti. J, Huszenicza Gy, Molnár L, and Horkai B. Peripartal changes in some metabolic parameters of blood and their prognostic value on postpartum chance of reconception (in German). Deutsch Tierärztl Wschr, 1982; 89: 357-361.

Hardie EM, and Kruse-Elliot K. Endotoxin shock. a review of causes. J Vet Int Med 1990; 4: 258-266.

Harmon RJ and Torre RM. Copper and zinc. Do they influence mastitis? Proc 33rd Annu Mtg Natl Mastitis Counc, Inc, Orlando, FL Natl Mastitis Counc, Inc, Arlington VA 1994; p 54

Harmon RJ, Clark TW, Trammell DS, Smith BA, Torre PM, and Hemken RW. Influence of copper status in heifers on response to intramammary chalenge with Escherichia coli endotoxin. J Dairy Sci 1994; 77(Suppl 1): 198 (Abstr.)

Harris TG, Battaglia DF, Brown ME, Brown MB, Carlson NE, Viguie C, Williams CY, and Karsch FJ. Prostaglandins mediate the endotoxin-induced suppression of pulsatile gonadotropin-releasing hormone and luteinizing hormone secretion in the ewe. Endocrin Philadelph 2000; 141: 1050-1058.

Hashimoto H, Igarashi N, Miyawaki T, and Sato T. Effects of TNF-α, IL-1β, and IL-6 on type-I iodothyronine 5'-deiodination in rat thyroid cell line, FRTL-5J Interferone. Cytokine Res 1995;15: 367-375.

Hassan Z, Daniel RCW, O'Boyle D, and Frost AJ. Effects of dry cow intramammary therapy on quarter infections in the dry period. Vet Rec 1999; 145: 635-639.

Hetzel H. Infectious catarhal mastitis of the dairy cows. (in Hungarian) Állatorvosi Lapok 1929; 52: 111-114; 124-126; 134-136.

Hirvonen J, Eklund K, Teppo AM, Huszenicza G, Kulcsar M, Saloniemi H, and Pyörälä S. Acute phase response in dairy cows with experimentally induced Escherichia coli mastitis. Acta Vet Scand 1999; 40: 35-46

Hockett ME, Hopkins FM, Lewis MJ, Saxton A,M, Dowlen HH, Oliver SP, and Schrick FN. Endocrine profiles of dairy cows following experimentally induced clinical mastitis during early lactation. Anim Reprod Sci 2000; 58: 241-251.

Hodges RT, Holland JTS, Neilson FJA, and Wallace NM. *Prototheca zopfii* in a herd of dairy cows. New Zealand Vet J 1985; 33: 108–111.

Hoeben D, Burvenich C, Massart-Leen AM, Lenjou M, Nijs G, Van Bockstaele D, and Beckers JF. In vitro effect of ketone bodies, glucocorticosteroidsand bovine pregnancy-associated glycoprotein on cultures of bone marrow progenitor cells of cows, and calves. Vet Immunol Immunopathol 1999; 68: 229-240.

Hoeben D, Heynemann R, and Burvenich C. Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect of respiratory burst activity of neutrophils. Vet Immunol Immunopathol 1997; 58: 165-170.

Hogan JS, Smith KL, Hoblet KH, Schoenberger PS, Todhunter DA, Hueston WD, Pritchard DE, Bowman GL, Heider LE, Brockett BL, and Conrad HR. Field survey of clinical mastitis in low somatic cell count herds. J Dairy Sci 1989; 72: 1547-1556.

Holst H, and Kindahl H. Hematological and blood biochemical effects of fasting and subsequent oral administration of endotoxin in prepubertal gilts. Acta Vet Scand 1995; 36: 499-508.

Holtenius P, and Traven M. Impaired glucose tolerance and heterogeneity of insulin responses in cows with abomasal displacement. J Vet Med A 1990; 37: 445-451.

Holtenius P, Olsson G, and Björkman C. Periparturient concentrations of insulin, glucagon and ketone bodies in dairy cows fed two different levels of nutrition and varying concentrate/roughage ratios. J Vet Med A 1993; 40: 118-127.

Holtenius P, Olsson G, Emanuelson M, and Wiktorson H. Effects of different energy levels, concentrate/forage ratios and lipid supplementation to the diet on the adaptation of the energy metabolism at calving in dairy cows. J Vet Med A 1996; 43: 427-435.

Honkanen-Buzalski T, and Seuna E. Isolation and identification of pathogens from milk In. Sandholm, M, Honkanen-Buzalski, T, Kaartinen, L, Pyörälä, S (eds). The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine, 1995; pp 121-142.

Hoshino S, Wakita M, Kobayashi Y, Sakauchi R, Nishiguchi Y, Ozawa A, Hodate K, Hamaguchi I, and Yotani Y. Variations in serum levels of insulin-like growth factor-I,

growth hormone and thyroid hormones during lactation in dairy cows. Comp Biochem Physiol 1991; 99A: 61-64.
Hossner KL, McCusker RH, and Dodson MV. Insulin-like growth factors and their binding proteins in domestic animals. Anim Sci 1997; 64: l-15.
Houseknecht KL, Baile CA, Matteri RL, and Spurlock ME. The biology of leptin. a review. J Anim Sci 1998; 76: 1405-1420.
Hove K. Insulin secretion in lactating cows. responses to glucose infused intravenously in normal, ketotic and starved animals. J Dairy Sci 1978; 61: 1407-1413.
Huhtanen P, Miettinen H, and Ylinen M. Effect of increasing ruminal butyrate on milk yield and blood constituents in dairy cows fed a grass silage-based diet. J Dairy Sci 1993; 76: 114-1124.
Hurley WL, Convey EM, Leung K, Edgerton LA, and Hemken RW. Bovine prolactin, TSH, T4 and T3 concentrations as affected by tall fescue summer toxicosis and temperature. J Anim Sci 1981; 51: 374-379.
Huszenicza Gy, and Stollár Zs. Possibilities for diagnosis of subclinical mastitis: a review (in Hungarian) Magy Áo Lapja 1993; 48: 460-465.
Huszenicza Gy, Haraszti J, Molnár L, Solti L, Fekete S, Ekés K, and Yaro AC. Some metabolic characteristics of dairy cows with different postpartum ovarian function. J Vet Med A 1988; 35: 506-515.
Huszenicza Gy, Kégl T, Kulcsár M, Oláh B, Gacs M, Oppel K, Stollár Zs, Jonsson P, Jánosi Sz. Diagnostic value of certain mastitis markers in following up the clinical and bacteriological changes in pharmacotherapeutic studies. Acta Vet Hung 1997; 45: 409-416.
Huszenicza Gy,. Jánosi Sz, Kulcsar M, Kóródi P, Dieleman S. J, Bartyik. J, and Ribiczei-Szabó P. Gram negative mastitis in early lactation may interfere with ovarian and certain endocrine functions and metabolism in dairy cows. Reprod Dom Anim 1998; 33: 147-153.
Hydbring E, Madej A, MacDonald E, Drugge-Boholm G, Berglund B, and Olsson K. Hormonal changes during parturition in heifers and goats related to the phases and severity of labour. J Endocrinol 1999;160: 75-85.
Jackson JA, Schuster DE, Silvia WJ, and Harmon R.J. Physiological responses to intramammary or intravenous treatment with endotoxin in lactating dairy cows. J Dairy Sci 1990; 73: 627-632.
Janan. J, Rudas P, Bartha T, Bozó S, and Gábor Gy. Effect of severe energy restriction and refeeding on thyroid hormones in bulls. Acta Vet Hung 1995; 43: 173-177.
Jarlov N, Andersen PH, and Hesselholt M. Pathophysiology of experimental bovine endotoxicosis: endotoxin induced synthesis of prostaglandins and thromboxane and the modulatory effect of some non-steroidal antiinflammatory drugs. Acta Vet Scand 1992; 33: 1-8.
Jensen HE, Aalbaek B, Bloch B, and Huda A. Bovine mammary protothecosis due to *Prototheca* zopfii. Med Mycol 1998; 36: 2, 89-95.
Johansson T, Funke H, Emanuelson U, and Saran A. Systemic treatment of chronic subclinical Staphylococcus aureus mastitis at drying off. Proc. 3rd Int. Mast. Sem. Tel-Aviv, Israel. 1995; Book 2. s-5, 54-57.

Jonsson P. Scandinavian system of the bacteriological diagnosis and aimed antimicrobial treatment of mastitis (in Hungarian). Magy Áo Lapja 1993; 48: 467-471.
Juvancz I, and Paksy A. Medical biometry (in Hungarian). Medicina Press, Budapest, Hungary 1982.
Kahl S, Capuco AV, Binelli M, Wanderkooi WK, Tucker HA, and Mosely WM. Comparison of growth hormone releasing factor and somatotropin. thyroid status of lactating, primiparous cows. J Dairy Sci 1995; 78: 2150-2158.
Kahl S, Elsasser TH, and Blum JW. Effect of endotoxin challenge on hepatic 5'-deiodinase activity in cattle. Dom Anim Endocr 2000; 18: 133-143.

Kandefer-Szerszen M, Filar J, Szuster-Ciesielska A, and Rzeski W. Suppression of interferon response of bovine leukcytes during clinical, and subclinical ketosis in lactating cows.(in German) Dtsch Tieraeztl Wochenschr 1992; 99: 440-443.

Kaplan MM. Regulatory influences on iodothyronine deiodination in animal tissues In. Hennemann, G (ed). Thyroid hormone metabolism. M Dekker Inc, New York, USA, 1986; pp 231-253.

Kapp P, Pethes Gy, Zsiros M, and Schuszter Z. Data on the pathogenesis of fatty liver disease in high-producing dairy cows (in Hungarian). Magy Áo Lapja, 1979; 34: 458-461.

Katholm J, and Andersen PH. Acute coliform mastitis in dairy cows: endotoxin and biochemical changes in plasma and colony-forming units. Vet Rec 1992; 131: 513-514.

Katoch RC, Nagal KB, Mandeep-Sharma, and Sharma N. *Protothecal* mastitis in a cow. A report. Indian J Anim Sci 1997; 67: 4, 292-293.

Kenison DC, Elsasser TH, and Fayer R. Tumor necrosis factor as a potential mediator of acute metabolice and hormonal responses to endotoxaemia in calves. Am J Vet Res 1991; 52: 1320-1326.

Kesler DJ,. Johnson HD, and Garverick HA. Postpartum concentrations of thyroxin in plasma of dairy cows. J Dairy Sci 1981; 64:1618-1620.

Kim T, Suh KS, Chae YS, and Kim YJ. Successful treatment with fluconazole of protothecosis developing at the site of an intralesional corticosteroid injection. Br J Dermatol 1996; 135: 803–806.

Kincaid RL, Hodson AS, Riley RE and Cronrath JD. Supplementation of diets for lactating cows with zinc as zinc oxide and zinc methionine. J Dairy Sci 1984; 67 (Suppl 1). 103 (Abstr)

Kindahl H, Daels P, Odensvik K, Daunt D, Fredricksson G, Stabenfeldt G, and Hughes JP. Experimental models of endotoxaemia related to abortion in the mare. J Reprod Fert (Suppl.) 1991; 44: 509-516.

Kindahl H, Odensvik K, Aiumlamai S, and Fredriksson G. Utero-ovarian relationships during the bovine postpartum period. Anim Reprod Sci 1992; 28: 363-369.

Kindahl H, Odensvik K, Bekana M, and Kask K. Prostaglandin release as a mediator between infections and impaired reproductive performance. Reprod Dom Anim 1996; 31: 441-444.

Kinsbergen M, Bruckmaier RM, and Blum JW. Metabolic, endocrine and haematological responses to intravenous E coli endotoxin administration in 1-week-old calves. J Vet Med A 1994; 41:530-547.

Kirk JH. Diagnosis and treatment of difficult mastitis cases. Agri-Practice 1991; 12: 15–20.

Klassing KC. Nutritional aspects of leucocytic cytokines. J Nutr 1988; 118:1436-1446 .

Klein K, Fuchs J, Kulapongs P, Mertz G, Suskind RM, and Olson RE. Endotoxemia in protein-energy malnutrition. J Pediatr Gastroenterol Nutr 1988; 7: 225-228.

Kleinbaum DG, and Kupper LL. Applied regression analysis and other multivariable methods. Duxbury Press, North Scituate, MA, US A1978.

Klucinski, W, Miernik-Degorska E, Degorski A, Targowski S, and Winnicka A. Effect of ketone bodies on the mitogenic response of bovine milk lymphocytes. J Vet Med A 1988a; 35: 626-631 .

Klucinski W, Degorski W, Miernik-Degorska E, Targowski S, and Winnicka A. Effect of ketone bodies on the phagocytic activity of bovien milk macrophages, and polymorphonuclear leukocytes. J Vet Med A 1988b; 35: 632-639.

Kocsár L, Bertók L, and Várterész V. Effect of bile acids on the intestinal absorption of endotoxins in rats. J Bact 1969; 100: 220-223-

Kolk JH van der, Breukink HJ, Wensing Th, and Mol. JA. Adrenocortical function testing in dairy cows and its effect on milk yield. Vet Quart 1991; 13: 144-147.

Kolk JH. van der. Some aspects of bovine pituitary-adrenocortical axis with special reference to persistent udder oedema. PhD. Dissertation. Faculty of Veterinary Medicine, Utrecht 1991

Kremer WDJ, Burvenich C, Noordhuizen-Stassen EN, Grommers FJ, Schukken YH, Heeringa R, and Brand A. Severity of experimental Escherichia coli mastitis in ketonemic, and nonketonemic dairy cows. J Dairy Sci 1993a; 76: 3428-3436.

Kremer WDJ, Noordhuizen-Stassen EN, Grommers FJ, Daemen AJJM, Henricks PAJ, Burvenich C, and Brand A. Preinfection chemotactic response of blood polymorphonuclear leukocytes to predict severity of Escherichia coli bovine mastitis. J Dairy Sci 1993b; 76: 2514-2518.

Krüger W. Data to the knowledges of an organism from the mucous secretions of broadleaves threes. Parts I and II. (in German) Zopf's Beiträge Physiologie und Morphologie niederen der Organismen (Leipzig) 1894; 4: 69–116.

Kulcsár M, Huszenicza Gy, Thuróczy. J, Szakáll I, Fekete S, Csillik Z, and Szilágyi M. Interrelationships between the ACTH-stimulated cortisol release and metabolic and reproductive patterns of dairy cows in the postpartum period. Proc of VIIth Congr of Internat Soc for Animal Clinical Biochemistry Glasgow, 1996; p 72

Kunz PL, and Blum. JW. Relationships between energy balances and blood levels of hormones and metabolites in dairy cows during late pregnancy and early lactation. Z Tierphysiol Tierernähr g u Futtermittelkde 1985; 54: 239-248.

Kushibiki S, Hodate K, Shingu H, Ueda Y, Mori Y, Itoh T, and Yokomizo Y. Effects of long-term administration of recombinant bovine tumor necrosis factor-α on glucose metabolism and growth hormone secretion in steers. Am J Vet Res 2001b; 62 :794-798.

Kushibiki S, Hodate K, Shingu H, Ueda Y, Shinoda M, Mori Y, Itoh T, and Yokomizo Y. Insulin resistance induced in dairy steers by tumor necrosis factor alpha is partially reversed by 2,4-thiazolidine-dione. Dom Anim Endocrinol 2001a; 21 :25-37.

Kushibiki S, Hodate, K, Ueda Y, Shingu H, Mori Y, Itoh T, and Yokomizo Y. Administration of recombinant bovine tumor necrosis factor-α affects intermediary metabolism and insulin and growth hormone secretion in dairy heifers. J Anim Sci 2000; 78:2164-2171.

Kuttin ES, Müller J, Feldman M, Neriya A, Uchovsky D, and Shahar A. Protothecosis in Israel. Israel J Vet Med 1986; 42: 1, 49.

Lagneau PE. First isolation of *Prototheca* zopfii in bovine mastitis in Belgium.(in French) J de Mycologie Medicale 1996; 6: 3, 145-148.

Lam TJGM, Van Vliet JH, Schukken YH, Grommers FJ, Van Velden.Russcher A, Barkema HW, and Brand A. The effect of discontinuation of postmilking teat disinfection in low somatoc cell count herds. 1. Incidence of clinical mastitis. Vet Quart 1997; 19: 41-47.

Lee SW, Tsou AP, Chan H, Thomas. J, Petire K, Eugui EM, and Alison AC. Glucocorticoids selectively inhibit the transcription of the interleukin-lβ gene and decrease the stability of' interleukin-lβ mRNA Proc Natl Acad Sci USA, l988; 85:1204-1208 .

Leininger MT, Portocarrero CP, Schinckel AP, Spurlock ME, Bidwell CA, Nielsen KL, and Houseknecht KL. Physiological response to acute endotoxemia in swine. effect of genotype on energy metabolism and leptin. Dom Anim Endocrinol 2000; 18:71-82.

Leonard. JL, and Visser TJ. Biochemistry of deiodination In. Hennemann, G (ed). Thyroid hormone metabolism. M Dekker Inc, New York, USA, 1986; pp 189-229.

Lerche M. A case of bovine mastitis caused by alga (*Prototheca*). (in German) Berl Münch Tierärztl Wschr 1952; 65: 64–69.

Liao W, Rudling M, and Angelin B. Contrasting effects of growth hormone and insulin-like growth factor I on the biological activities of endotoxin in the rat. Endocrinol 1997; l38: 289-295 .

Linquist WE. *Prototheca* mastitis—a case report. 20th Annual Meeting. National Mastitis Council, Inc. 1981; 161-163.

Lohuis JACM. Escherichia coli mastitis in the cow. Some aspects of symptomatic treatment and defense mechanisms. PhD. Dissertation. Faculty of Veterinary Medicine, Utrecht1989;

Lohuis. JACM, Verheijden JHM, Burvenich C, and Miert van ASJPAM. Pathophysiological effects of endotoxin in ruminants 2 Metabolic aspects. Vet Quart 1988; 10:117-125.

Markus, G. Data on Hungarian udder health conditions, based on field experiences (In Hungarian) In. Simon F, Szita G, Merényi I (ed/szerk). Tőgyegészség és tejminőség (Udder health and milk quality) Mezőgazda Kiadó, Budapest 2000.

Markusfeld O. Relationship between overfeeding, metritis and ketosis in high yielding dairy cows. Vet Rec 1985; 116: 489-491.

Mattila T. Diagnostic problems in bovine mastitis. Thesis. College of Veterinary Medicine, Helsinki, Finland. 1985.

McCusker RH. Controlling insulin-like growth factor activity and the modulation of insulin-like growth factor binding protein and receptor binding. J Dairy Sci 1998; 81: 1790-1800 .

McDonald JS, Richard JL, and Anderson AJ. Antimicrobial susceptibility of *Prototheca zopfii* isolated from bovine intramammary infections. Am J Vet Res 1984a; 45: 1079–1080.

McDonald JS, Richard JL, and Cheville NF. Natural and experimental bovine intramammary infection with *Prototheca zopfii*. Am J Vet Res 1984b; 45: 592–595.

McGuire MA, Bauman DE, Dwyer DA, and Cohick WS. Nutritional modulation of the somatotropin/ insulin-like growth factor system. response to feed deprivation in lactating cows. J Nutr 1995a ;125:493-502.

McGuire MA, Dywer DA, Harrell RJ, and Bauman DE. Insulin regulates circulating insulin-like growth factors and some of their binding proteins in lactating cows. Amer Can J Physiol 1995b; 269: E723-730 .

McGuire MA, Vicini. JL, Bauman DE, and Veenhuizen. JJ. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J Anim Sci 1992; 70: 2901-2910 .

McMahon CD, Elsasser TH, Gunter DR, Sanders LG, Steele BP, and Sartin L. Estradiol/progesterone implants increase food intake, reduce hyperglycemia and increase insulin resistance in endotoxic steers. J Endocrinol 1998; 159:469-478 .

Meek AH, Barnum DA, and Newbould FHS. Use of total and differential somatic cell counts to differentiate potentially infected from potentially non-infected quarters and cows and between herds of various levels of infection. J Food Prot 1980; 43: 10-14.

Memon RA, Feingold KR, Moser AH, Doerrler W, Adi S, Dinarello CA, and Grunfeld C. Differential effects of interleukin-1 and tumor necrosis factor on ketogenezis. Am J Physiol 1992; 263:E301-E309.

Miltenburg. JD, de Lange D, Crauwels APP, Bongers JH, Tielen MJM, Schukken YH, and Elbers ARW, Incidence of clinical mastitis in a random sample of dairy herds in the southern Netherlands. Vet Rec 1996; 139: 204-207.

Mohabeer AJ, Kaplan PJ, Southern jr PM, and Gander RM. Algaemia due to *Prototheca wickerhamii* in a patient with myasthenia gravis. J Clin Microbiol 1997; 35: 3305–3307.

Mol J, and Rijnberk A. Pituitary function In. Kaneko,. JJ, Harvey,. JW, Bruss, M L (eds). Clinical biochemistry of domestic animals. Academic Press, San Diego, London, Boston, New York, Sydney, Tokio, Toronto, 1997; 517-551.

Moore DA, Cullor JS, Bondurant RH, and Sicho WM. Preliminary field evidence for the association of clinical mastitis with altered interestrus intervals in dairy cattle. Theriogenology 1991; 36: 257-265.

Moore FM, Schmidt GM, Desai D, and Chandler FW. Unsuccessful treatment of disseminated protothecosis in a dog. J Am Vet Med Assoc 1985; 186: 705–708.

Müller W. Aggregationstest as a fast methode to differentiate the genus *Prototheca* from different species of the genus *Candida* (Short communications) (in German). Mh Vet Med

1988; 43: 278-279.
N R C. Nutrient requirements of dairy cattle. 6th rev ed Natl Acad Sci Washington, DC. 1989.
Nagy P, Solti L, Kulcsár M, Reiczigel J, Huszenicza Gy, Abaváry-Mihály K, and Wöfling A. Progesterone determination in equine plasma using different immunoassays. Acta Vet Hung 1998; 46:501-513 .
Nagy UZs, Csaba G, and Bertók L. Effects of thyrotropin (TSH) treatment on the responsiveness of the thyroid gland during endotoxib shock in adult rats. Acta Physiol Hung 1983; 62:187-190.
Nap RC, Mol. JA, and Hazewinkel HAW. Age-related plasma concentrations of growth hormone and insulin-like growth factor-1 in great dane pups fed different dietary levels of protein. Dom Anim Endocr 1993; 10: 237-247.
Natzke RP, Everett RW, and Bray DR. Effect of drying off practices on mastitis infection. J Dairy Sci 1975; 58: 1818-1827.
Natzke RP, Everett RW, Guthrie RS, Keown JF, Meek AM, Merrill WG, Roberts SJ, and Schmidt GH. Mastitis control program: effect on milk production. J Dairy Sci 1972; 55: 1256-1260.
Natzke RP. Elements of mastitis control. J Dairy Sci 1981; 64: 1431-1442.
Natzke RP. Therapy: one component in a mastitis control system. J Dairy Sci 1971; 54: 1895-1901.
Neave F K, Dodd FH, and Kingvill RG. A method of controlling udder disease. Vet Rec 1966; 78: 521-522.
Neave FK, and Oliver J. The relationship between the number of mastitis pathogens placed on the teats of dry cows, their survival, and the amount of intramammary infection caused. J Dairy Res 1962; 29: 79-93.
Neave FK, Dodd FH, and Henriques E. Udder infections in the dry period. J Dairy Res 1950; 17: 37-49.
Neave FK, Dodd FH, Kingwill RG, and Westgarth DR. Control of mastitis in the dairy herd by hygiene and management. J Dairy Sci 1969; 52: 696-707.
Neijenhuis F, Barkema HW, Hogeveen H, and Noordhuizen JPTM. Relationship between teat-end callosity and occurrence of clinical mastitis. J Dairy Sci 2001; 84: 2664-2672.
Newsholme P, Curi R, Gordon S and Newsholme EA. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. Biochem J 1986; 239: 121-125.
Newsholme P, Gordon S and Newsholme EA. Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. Biochem J 1987; 242: 631-636.
Nickerson SC, and Akers RM. Effects of prepartum blockade of microtubule formation on ultrastructural differentiation of mammary epithelium in Holstein heifers. Int J Biochem 1983; 15: 771-775.
Nikolić JA, Nedić O, and Masnikosa R. Determination of insulin-like growth factor-II in human serum. J Serb Chem Soc 1998; 63:805-815.
Nikolic JA, Nedic O, Samanch H, Aleksic S, Miscevic B, and Kulcsár M. Pripheral circulating insulin-like growth factor-I and II in cattle. Acta Vet Hung, 2001a;. 49: 53-63.
Nikolić JA, Ratković M, and Nedić O. Determination of insulin-like growth factor-I by radioimmunoassay. J Serb Chem Soc 1996; 61:1149-1157.
Nikolić JA, Šamanc H, Kovačević M, Bugarski D, and Masnikosa R. Serum concentrations of insulin-like growth factors and thyroid hormones in healthy and ketotoic dairy cows during the puerperium. Acta Vet Beograd 2001b; 51:73-88.
Noma A, Okabe H, and Kita M. Colorimetric microdetermination of non-esterified fatty acids in diabetic ketosis. Clin Chem Acta 1973; 43: 314-316.

Oliver SP, and Maki JL. Persistence of antibiotic residues in mammary secretions during the nonlactating period following intramammary infusion at drying off. J Dairy Sci 1987; 70(Suppl. 1): 163. (Abstr.)

Oliver SP, and Sordillo LM. Approaches to the manipulation of mammary involution. J Dairy Sci 1989; 72: 1647-1664.

Oliver SP, and Sordillo LM. Udder health in the periparturient period. J Dairy Sci 1988; 71: 2584-2606.

Oliver SP, Schrick FN, Hockett ME, and Dowlen HH. Clinical and subclinical mastitis during early lactation impairs reproductive performance of dairy cows. Proc of National Mastitis Council, Inc Regional Meeting (Cleveland, Ohio, 24 Aug, 2000), 2000; pp 34-51.

Oliver SP. Importance of the dry period in the control of intramammary infections by environmental mastitis pathogens. Proc. 26th Annu. Mtg. Natl. Mastitis Counc. Arlington, VA. 1987; P. 81.

Oltenacu PA, and Ekesbo I. Epidemiological study of clinical mastitis in dairy cattle. Vet Res 1994; 25: 208-212.

Osteras O, and Sandvik L. Effects of selective dry cow therapy on culling rate, clinical mastitis, milk yield and somatic cell count. A randomized clinical field study in cows. J Vet Med B 1996; 43: 555-575.

Osteras O, Aursjo J, Gjul GG, Jorstad A, and Gronningsaeter-Gjul G. Effect of dry cow therapy on subclinical mastitis- an evaluation of long-acting and short-acting intramammaria. J Vet Med B 1994; 41: 529-540.

Osteras O, Edge VL, and Martin SW. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J Dairy Sci 1999a; 82: 1221-1231.

Osteras O, Martin SW, and Edge VL. Possible risk factors associated with penicillin-resistant strains of Staphylococcus aureus from bovine subclinical mastitis in early lactation. J Dairy Sci 1999b; 82: 927-938.

Osteras O, Sandvik L, Aursjo J, Gjul GG, and Jorstad A. Assessment of strategy in selective dry cow therapy for mastitis control. J Vet Med B 1991; 38: 513-522.

Owens WE, Ray CH, Watts JL, and Yancey RJ. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. J Dairy Sci 1997; 80: 313-317.

Paape MJ, Carroll DW, Kral AJ, Miller RH, and Desjardins C. Corticosteroids, circulating leukocytes and erythrocytes in cattle. diurnal changes and effects of bacteriologic status, stage of lactation and milk yield on response to adrenocorticotropin. Am J Vet Res 1974; 35: 355-362.

Pang X-P, Hershman JM, Mirell CJ, and Pakery AE. Impairment of hypothalamic-pituitary-thyroid function in rats treated with human recombinant tumor necrosis factor-α (cachectin). Endocrinol 1989; 125:76-83.

Pankey JW, Barker RM, Twomey A, and Duirs G. Comparative efficacy of dry cow treatment regimens against Staphylococcus aureus. New Zealand Vet J 1982; 30: 13-15.

Persson K, Larsson I, and Hallen SC. Effects of certain infalmmatory mediators on bovine neutophil migration in vivo, and in vitro, Vet Immunol Immunopathol 1993;37: 99-112.

Persson-Waller K. Modulation of endotoxin-induced inflammation in the bovine teat using antagonists/inhibitors to leukotrienes, platelet activating factos, and interleukin-1 beta. Vet Immunol Immunopathol 1997;57: 239-251.

Peter AT, and Bosu WTK. Effects of intrauterine infections on the function of the corpora lutea formed after first postpartum ovulations in dairy cows. Theriogenology 1987; 27: 593-609.

Peter AT, and Bosu WTK. Relationship of uterine infections and folliculogenesis in dairy cows during early puerperium. Theriogenology 1988; 30: 1045-1051.

Peter AT, Bosu WTK, and DeDecker RJ. Suppression of preovulatory LH surges in heifers after intrauterine infusions of Escherichia coli endotoxin. Am J Vet Res 1989; 50: 368-373.

Peter AT, Bosu WTK, and Gilbert RO. Absorption of Escherichia coli endotoxin (lipopolysaccharide) from the uteri of postpartum dairy cows. Theriogenology 1990; 33: 1011-1014.

Peter AT, Bosu WTK, and Luke CW. Plasma endotoxin and concentration of stable metabolites of prostacyclin, thromboxane A_2 and prostaglandin E_2 in postpartum dairy cows. Prostaglandins 1987; 34: 15-28.

Pethes Gy, Bokori. J, Rudas P, Frenyó VL, and Fekete S. Thyroxin, triiodothyronine, reverse-triiodothyronine and other physiological characteristics of periparturient cows fed restricted energy. J Dairy Sci 1985; 68: 1148-1154.

Philpot WN. Control of mastitis by hygiene and therapy. J Dairy Sci 1979; 62: 168-176.

Pore RS, and Shahan TA. *Prototheca zopfii*: natural, transient occurrence in pigs and rats. Mycopathol 1988; 101: 85–88.

Pore RS, Barnett FA, Barnes jr WC, and Walker JD. *Prototheca* ecology. Mycopathol 1983; 81: 49–62.

Pore RS, Shahan TA, and Pore MD. Occurrence of *Prototheca zopfii*, a mastitis pathogen in milk. Vet Microbiol 1987; 15: 315–324.

Pore RS. *Prototheca* taxonomy. Mycopathol 1985; 90: 29–139.

Pore RS. Selective medium for the isolation of *Prototheca*. Appl Microbiol 1973; 26: 648–649.

Postle DS, and Natzke RP. Efficacy of antibiotic treatment in the bovine udder. Vet Med Small Anim Clin Dec 1974; 69:1535-1539.

Poutrel B, and Rainard P. California Mastitis Test guide of selective dry cow therapy. J Dairy Sci 1981; 64: 241-248.

Preisler MT, Weber PSD, Tempelman RJ, Erskine RJ, Hunt H, and Burton JL. Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. Am J Vet Res 2000; 61:14-19.

Pyörälä S, and Pyörälä EO. Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows: 487 cases (1989-1995). J Am Vet Med Assoc 1998; 212: 407-412..

Pyörälä S, Pyörälä EO, and Trenti F. Efficacy of systemic therapy in bovine clinical mastitis during lactation. Proc. 18th World Buiatrics Cong. Bologna, Italy. 1994; Vol.1: 571-574.

Pyörälä S. Staphylococcal and Streptococcal mastitis. In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, and Pyörälä S. (eds): The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine, 1995a; p. 143-148.

Pyörälä S. Therapy of clinical mastitis. In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, and Pyörälä S. (eds): The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine, 1995b; p. 201-208.

Pyörälä S, and Syvajarvi. J. Bovine acute Mastitis. Part I. Clinical aspects and parameters of inflammation in mastitis caused by different pathogens. J Vet Med B 1987; 34: 573-584.

Quinn PJ, Carter ME, Markey B, and Carter GR. Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited Publ, London 1994.

Rajaram S, Baylink DJ, and Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids. regulation and functions. Endocrine Rev 1997; 18: 801-831 .

Ravin HA, Rowley D, Jenkind C, and Fine J. On absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal. J Exp Med 1960; 112: 783-792.

Rendos JJ, Eberhart RJ, and Kesler EM. Microbial populations of teat ends of dairy cows and bedding materials. J Dairy Sci 1975; 58: 1492-1500.

Rhone Merieux (ed.): Control and treatment of bovine mastitis. Spiramycin: the outstanding antibiotic. Manufacturer's manual. Paris. 1991.

Riis PM, and Madsen A. Thyroxin concentrations and secretion rates in relation to pregnancy, lactation and energy balance in goats. J Endocrinol 1985; 107:421-427.

Rivier C, and Rivest S. Effect of stress on the activity of the hypothalamic - pituitary - gonadal axis. peripheral and central mechanisms. Biol Reprod 1991; 45: 523-532.

Roets E, Burvenich C, Diez-Fraile A, and Noordhuizen-Stassen EN. Evaluation of the role of endotoxin, and cortisol on modulation of CD18 adhesion receptors in cows with mastitis caused by Escherichia coli. Am J Vet Res 1999; 60: 534-540.

Romo GA, Elsasser TH, Kahl S, Erdman RA, and Casper DP. Dietary fatty acids modulate hormonal responses in lactating cows. mechanistic role for 5'deiodinase activity in tissue. Dom Anim Endocrinol 1997; 14:409-420.

Ronge H, Blum. J, Clement C,. Jans F, Leuenberger H, and Binder H, Somatomedin C in dairy cows related to energy and protein supply and to milk production. Anim Prod 1988; 47: 165-183.

Rumsey TS, McLeod K, Elsasser TH, Kahl S, and Baldwin RL. Effects of oral chlortetracycline and dietary protein level on plasma concentrations of growth hormone and thyroid hormones in beef steers before and after challenge with a combination of thyrotropin-releasing hormone and growth hormone releasing hormone. J Anim Sci 1999; 77:2079-2087.

Russell-Jones DL, and Umpleby M Protein anabolic action of insulin, growth hormone and insulin-like growth factor I. European. J Endocrinol 1996; 135: 631-642 .

Salmon WD, and Daughaday WH. A hormonally controlled serum factor which stimulates sulphate incorporation by cartilage in vitro. J Lab Clin Med 1957; 49: 825-836 .

Sandholm M, Ali-Vehmas T, Nyholm K, Honkanen-Buzalski T, and Louhi M: Failure mechanisms in lactational therapy of staphylococcal mastitis. Flem Vet J 1991; 62: 171-186.

Sandholm M, and Pyörälä S. Coliform mastitis. In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, and Pyörälä S. (eds): The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine 1995a; p. 149-160.

Sandholm M, and Pyörälä S. Dry cow therapy In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, Pyörälä S. (eds): The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine; 1995b. p. 209-214.

Sandholm M, Kaartinen L, and Pyörälä S. Bovine mastitis—why does antibiotic therapy not always work? An overview. J Vet Pharmacol Ther 1990; 13: 248-260.

Sandholm M. A critical view on antibacterial mastitis therapy. In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, and Pyörälä S. (eds): The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine, 1995a; p. 169-186.

Sandholm M. Inflammation in mastitis. In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, and Pyörälä S. (eds): The bovine udder and mastitis. University of Helsinki, Faculty of Veterinary Medicine, 1995b; p. 59-75.

Sartin JL, Elsasser TH, Gunter DR, and McMahon CD. Endocrine modulation of physiological responses to catabolic disease. Dom Anim Endocrin 1998; 15: 423-429.

Sartorelli P, Paltrinieri S, and Agnes F. Non-specific immunity and ketone bodies I. In vitro studies on chemotaxis and phagocytosis of ovine neutrophils. J Vet Med A 1999; 46:613-619.

Sartorelli P, Paltrinieri S, and Comazi S. Non-specific immunity and ketone bodies II. In vitro studies on adherence and superoxide anion production in ovine neutrophils. J Vet Med A 2000; 47:1-8 .

Sato S, Suzuki T, and Okada K. Suppression of mitogenic response of bovine peripheral blood lymphocytes by ketone bodies. J Vet Med Sci 1995; 57: 183-185.

Schams D, Graf F, Graule B, Abele M, and Prokopp S. Hormonal changes during lactation in cows of three different breeds. Livestock Prod Sci 1991; 27:285-296.

Scherf H, Frye TM and Williams SN. Vitamin A and β-carotene. a nutritional approach to the control of mastitis in dairy cattle. Proc 33rd Annu Mtg Natl Mastitis Counc, Inc, Orlando, FL Natl Mastitis Counc, Inc, Arlington VA 1994; p 77.

Schlenstedt R, Zschock M, Kloppert B, and Wolter W. Occurrence of *Prototheca* mastitis in dairy farms in Hessen. (in German) Tierärztl Prax Ausg G 1997; 25: 407–412.

Schönborn C, Seffner W. Data to the pathology and microbiology of bovine mastitis caused by *Prototheca trispora*. (in German) Mh Vet Med 1977; 32: 685-693

Schukken YH, Erb HN, and Smith RD. The relationship between mastitis and retained placenta in a commercial population of Holstein dairy cows. Prev Vet Med 1988; 5: 181-190 .

Schukken YH, Grommers FJ, and van de Geer D, Brand A. Incidence of clinical mastitis on farm with low somatic cell counts in bulk milk. Vet Rec 1989; 125: 60-63.

Schukken YH, Leslie KE, Lam TJGM, and Sole J. Staphylococcus aureus: incidence,prevalence, and risk factors for intramammary infection. Proc. Natl. Mastitis Council Annu. Mtg. Madison, WI. 1993a; p. 19.

Schukken YH, Vanvliet J, Vandegeer D, and Grommers FJ. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J Dairy Sci 1993b; 76: 2925-2930.

Schultze WD. Effects of selective regimen of dry cow therapy on intramammary infection and on antibiotic sensitivity of surviving pathogens. J Dairy Sci 1983; 66: 892-903.

Schuster DE, and Harmon RJ. High cortisol concentrations and mediation of the hypogalactia during endotoxin-induced mastitis. J Dairy Sci 1992; 75: 739-746.

Schuster DE, Kehrli MEJr., Rainard P, and Paape M. Complement fragment C5a, and inflammatory cytokines in neutrophil recruitment during intramammary infection with Escherichia coli. Infect Immun 1997; 65: 3286-3292.

Schuster DE, Lee EK, and Kehrli MEJr. Bacterial growth, inflammatory cytokine production,, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation. Am J Vet Res 1996; 57: 1569-1575.

Schuster H, Blaschke-Hellmessen R. Epidemiology of the bovine protothecal mastitis: isolation of Prototheca alga from the environment of farm animals. (in German) Mh Vet Med 1983; 38: 24-29.

Seffner W. Prototheca-infections. (in German) In: Beer, J. (ed.) Infektionskrankheiten der Haustiere. VEB Gustav Fischer Verlag, Jena, 1987; p. 859–861.

Shahan TA, and Pore RS. *In vitro* susceptibility of *Prototheca* spp. to gentamicin. Antimicrob Agents Chemother 1991; 35: 2434–2435.

Sinkevich MG, Barto PB, Bush LJ, Wells ME, and Adams GD. Effectiveness of antibiotic infusion at drying off in preventing new mastitis infections in cows. Bovine Pract 1974; 9: 43-46.

Skaar TC, Vega. JR, Pyke SN. and Baumrucker CR. Changes in insulin-like growth factor-binding proteins in bovine mammary secretions associated with pregnancy and parturition. J Endocrinol 1991; 131:127-133.

Ślebodziński AB, Brzezińska-Ślebodzińska E, Styczyńska E, and Szejnoga M. Presence of thyroxin deiodinases in mammary gland. possible modulation of the enzyme-deiodinating activity by somatotropin. Dom Anim Endocrinol 1999; 17:161-169 .

Smith A, Dodd FH, and Neave FK. The effect of intrammary infection during the dry period on the milk production of the affected quarter at the start of the succeeding lactation. J Dairy Res 1968; 35: 287-290.

Smith A, Neave FK, Dodd FH, and Brander GC. Methods of reducing the incidence of udder infection in dry cows. Vet Rec 1966; 79: 233-236.

Smith A, Westgarth DR, Jones MR, Neave FK, Dodd FH, and Brander GC. Methods of reducing the incidence of udder infection in dry cows. Vet Rec 1967; 81: 504-510.

Smith BB, and Wagner WC. Effect of Escherichia coli endotoxin and thyrotropin-releasing hormone on prolactin in lactating sows. Am J Vet Res 1985; 46: 175-182.

Smith BB, and Wagner WC. Suppression of prolactin in pigs by Escherichia coli endotoxin. Science 1984; 224: 605-609.

Smith BP. Understanding the role of endotoxins in Gram negative septicemia. Vet Med 1986; 17: 1148-1161.

Smith KL, and Todhunter DA. The physiology of mammary glands during the dry period and the relationship to infection. Proc. 26th Annu. Mtg. Natl. Mastitis Counc. Arlington, VA.; 1982. P. 87-100.

Smith KL, Todhunter DA, and Schoeneberger PS. Environmental pathogens and intramammary infection during the dry period. J Dairy Sci 1985; 68: 402-417.

Smits E, Burvenich C, Guidry AJ and Roets E. In vitro expression of adhesion receptors and diapedesis by polymorphonuclear neutrophils during experimentally induced Streptococcus uberis mastitis. Infect Immun 1998; 66: 2529-2534.

Soback S, Ziv G, Winkler M, and Saran A. Systemic dry cow therapy – a preliminary report. J Dairy Sci 1990; 73, 661-666.

Sordillo LM, and Nickerson SC. Morphologic changes in the bovine mammary gland during involution and lactogenesis. Amer J Vet Res 1988; 49: 1112-1120

Sordillo LM, and Peel JE. Effect of interferon-γ on the production of tumor necrosis factor during acute Escherichia coli mastitis. J Dairy Sci 1992; 75: 2119-2125.

Sordillo LM, Oliver SP, and Nickerson SC. Caprine mammary differentiation and initiation of lactation following prepartum colchicine infusion. Int J Biochem 1984a; 16: 1265-1272.

Sordillo LM, Oliver SP, Duby RT, and Rufner R. Effects of colchicine on milk yield, composition, and cellular differentiation during caprine lactogenesis. Int J Biochem 1984b; 16: 1135-1141.

Spalton DE. Bovine mastitis caused by *Prototheca zopfii*: A case study. Vet Rec 1985; 116: 347-349.

Stabenfeldt GH, and Edqvist LE. Female reproductive processes. In: Swenson MJ. (ed): Dukes' physiology of domestic animals. Tenth edition. Cornell University Press, Ithaca, 1990; p. 798-832.

Steiger M, Senn M, Altreuther G, Werling D, Sutter F, Kreuzer M, and Langhans W. Effect of prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. J Anim Sci 1999; 77:2523-2532.

Sudman MS, and Kaplan W. Identification of the *Prototheca* species by immunofluorescence. Appl Microbiol 1973; 25: 981–990.

Suriyasathaporn W, Daemen AJJM and Schukken YH. Effect of serum β-hydroxybutyrate levels on in vitro chemotaxis of bovine leukocates. 10th international conference of production diseases in farm animals, 2124 August, Utrecht, The Netherlands, 1998; 266.

Suriyasathaporn W, Daemen AJJM, Noordhuizen-Stassen EN, Dieleman SJ, Nielen M, and Schukken YH. Beta-hydroxiybutyrate levels in peripheral blood, and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. Vet Immunol Immunopathol 1999; 68: 177-186.

Suriyasathaporn W, Heuer C, Noordhuizen-Stassen E, and Schukken YH. Hyperketonaemia and the impairement of udder defense. a review. Vet Res 2000; 31:397-412.

Suriyasathaporn, W. Negative energy balance in postpartum dairy cows: its effect on clinical mastitis and reproductive performance. Thesis. Faculty of Veterinary Medicine. Utrecht University. The Netherlands. 2000.

Suzuki C, Yoshioka K, Iwamura S, and Hirose H. Endotoxin induces delayed ovulation following endocrine aberration during the prooestrus phase in Holstein heifers. Dom Anim Endocrinol 2001; 20:267-278.

Svanberg E, Healey. J, and Mascarenhas D. Anabolic effects of rhIGF-I/IGFBP-3 in vivo are influenced by thyroid status. Eur J Clin Invest 2001; 31:329-336.

Swanson EW, and Poffenbarger JI. Mammary gland development of dairy heifers during their first gestation.J Dairy Sci 1979; 62: 702-714.

Taniyama H, Okamota F, Kurosawa T, Furuoka H, Kaji Y, Okada H, and Matsukawa K. Disseminated protothecosis caused by *Prototheca zopfii* in a cow. Vet Pathol 1994; 31: 123–125.

Taponen. J, Kulcsár M, Kátai L, Huszenicza Gy, Rodríguez-Martínez H, and Katila T. Short oestrous cycles and oestrous signs after premature ovulations induced with cloprostenol and gonadotropin-releasing hormone in cyclic dairy cows. Theriogenology, accepted for publication

Targowski SP, and Klucinski W. reduction in mitogenic response of bovine lymphocytes by ketone bodies. Am J Vet Res 1983; 44: 828-830.

Tenhagen BA, Kalbe P, Klunder G, Heuwieser W, Baumgärtner B. Tierindividuelle Risikofaktoren für die Protothekenmastitis des Rindes. Deutsche-Tierärztliche Wochenschrift 1999; 106: 376-380.

Thissen JP, Ketelslegers JM, and Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocrine Rev 1994; 15: 80-101 .

Thrift TA, Bernal A, Lewis AW, Neuendorff DA, Willard CC, and Randel RD. Effects of induced hypothyroidism or hyperthyroidism on growth and reproductive performance of Brahman heifers. J Anim Sci 1999; 77:1833-1843.

Thuroczy J, Huszenicza G, Kulcsar M, Fekete S, Szakall I, and Solti L. Clinical and reproductive consequences of temporary postpartum hypocorticism in dairy cows. Abstract. J Physiol Pharmacol (Suppl. 1) 1996; 47: 138.

Tiirats T. Thyroxin, triiodothyronine and reverse- triiodothyronine concentrations in blood plasma in relation to lactational stage, milk yield, energy and dietary protein intake in Estonian dairy cows. Acta Vet Scand 1997; 38:339-348.

Todhunter DA, Smith KL, and Schoeneberger DS. In vitro growth of coliform bacteria in mammary secretions. J Dairy Sci 1982; 65(Suppl. 1): 170. (Abstr.)

Torres E, Nakao T, Hiramune T, Moriyoshi M, Kawata K, and Nakada K. Stress and uterine bacterial flora in dairy cows following clinically normal and abnormal puerperium. J Rep Dev 1997a ; 43: 157-163.

Torres E, Nakao T, Osawa T, Moriyoshi M, Kawata K, and Nakada K. Postpartum adrenal, pituitary and ovarian functions in dairy cows. J Vet Med A 1997b ; 44: 133-142.

Tveit B, Lingaas,F, and Standal N. Thyroid function in heifers measured by hormone levels before and after injection of thyrotropin releasing hormone Methods, repeatability and correlation with production traits. Acta Agric Scand 1990; 40:175-181.

U. Nagy S, and Bertok L. Influence of experimentally induced endotoxemias on the thyroid function of rats. Acta Physiol Hung 1990; 76: 137-141.

U. Nagy S, Csaba G, and Bertok L. Effect of thyrotropin (TSH) treatment on the responsiveness of the thyroid gland during endotoxin shock in adult rats. Acta Physiol Hung 1983; 62: 187-190.

Urbán E. The main groups of antibiotics and their effect. (in Hungarian) In: Czirók É. (editor) Klinikai és járványügyi bakteriológia. Melania Kft. Budapest; 1999. p. 573.

Uribe HA, Kennedy BW, Martin SW, and Kelton DF. Genetic parameters for common health disorders of Holstein cows. J Dairy Sci 1995; 78: 421-430.

Valde JP, Hird DW, Thormond MC, and Osteras O. Comparison of ketosis, Clinical mastitis, somatic cell count, and reproductive performance between free stall and tie stall barns in Norwegian dairy herds with automatic feeding. Acta Vet Scand 1997; 38:181-192.

Van Veen HS, and Kremer WD. Mycotic mastitis in cattle. (in Dutch) Tijdschr Diergeneeskd 1992; 117: 414–416.

Van Werven T, Noordhuizen-Stassen EN, Daemen AJ, Schukken YH, Brand A, and Burvenich C. Preinfection in vitro chemotaxis, phagocytosis, oxidative burst, expression of CD11/CD18 receptors, and their predictive capacity on the outcome of mastitis induced in dairy cows with Escherichia coli. J Dairy Sci 1997; 80: 67-74.

Van Werven T. The role of leukocytes in bovine Escherichia coli mastitis. Ph.D. thesis. Utrecht's Diss. 1999;

VanDamme DM. Use of miconazole in treatment for bovine mastitis. Agri-Practice 1983; 9: 1425–1427.

VandeHaar MJ, Yousif G, Sharma BK, Herdt TH, Emery RS, Allen MS, and Liesman. JS. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. J of Dairy Sci 1999; 82: 1282-1295 .

Vega. JR, Gibson CA, Skaar TC, Hadsell DL, and Baumrucker CR. Insulin-like growth factor (IGF)-I and –II and IGF binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows. J Anim Sci 1991; 69:2538-2547.

Waage S. Plasma concentrations of cortisol in cows with hypocalcaemia in relation to their responses to treatment with calcium. Res Vet Sci 1984; 3: 164-168.

Walker PG. A colorimetric method for the estimation of acetoacetate Biochem. J 1954; 58: 699-700.

Wartofsky L, and Burman KD. Alterations in thyroid function in patients with systemic illnesses. the "euthyroid sick syndrome". Endocr Rev 1982; 3:164-217.

Wessels BC, Gaffin SL, and Wells MT. Circulating plasma endotoxin (lipopolysaccharide) concentrations in healthy and hemorrhagic enteritic dogs: antiendotoxin immunotherapy in hemorrhagic enteritic endotoxemia. J Am Anim Hosp Assoc 1987; 23: 291-295.

Westphal O. Bacterial endotoxins. Int Arch Allergy Appl Immun 1975; 49: 1-11.

Whitford HW, Rosenbusch RF, and Lauermann LH. Mycoplasmosis in Animals: Laboratory Diagnosis. Iowa State University Press, Ames, Iowa, USA. 1994; p. 17–19.

Wichtel. JJ, Craigie AL, Freeman DA, Varela-Alvarez H, and Williamson NB. Effect of selenium and iodine supplementation on growth rate and on thyroid and somatotropic function in dairy calves at pasture. J Dairy Sci 1996; 79:1865-1872.

Wilhelm A, Blaschke-Hellmessen R, Zieris H. Protothecal mastitis in cows. (in German) Prakt Tierarzt 1992; 74: 556-560.

Williamson JH, Woolford MW, and Day AM. The prophylactic effect of a dry-cow antibiotic against Streptococcus uberis. N Zealand Vet J 1995; 43: 228-234.

Yagoda CR, Bylund-Fellenius AC, and Kindahl H. Some effects of Gram negative bacterial endotoxin and its importance as a contaminator of biological preparations. Acta Vet Scand 1990; 31: 193-206.

Yamaguchi Y, Yamaguchi K, Babb JL, and Gans H. In vivo quantitation of the rat liver's ability to eliminate endotoxin from the portal blood. J Reticuloendothelial Soc 1982; 32: 409-422.

Yu AA, Kemppainen RJ, and McDonald JM. Effect of endotoxin on hormonal responses to thyrotropin and thyrotropin-releasing hormone in dogs. Am J Vet Res 1998; 59: 186-191.

Zadoks RN, Allore HG, Barkema HW, Sampimon OC, Wallenberg GJ, Gröhn YT, and Schukken YH. Cow- and quarter-level risk factors for Streptococcus uberis and Staphylococcus aureus mastitis. J Dairy Sci 2001; 84:2649-2663.

Zecconi A, Piccinini R, and Guarini CPB. Tylosin in cows in the dry period. (in Italian) Obiettivi e Documenti Veterinari, 1999; 20: 49-54.

Zerbe H, Schneider N, Leibold W, Wensing T, Kruip TAM, and Schuberth HJ. Altered functional and immunophenotypical properties of neutrophil granulocytes in postpartum cows associated with fatty liver. Theriogenology 2000; 54:771-786.

Ziv G, Storper M, and Saran A. Comparative efficacy of three antibiotic products for the treatment and prevention of subclinical mastitis during the dry period. Vet Quart 1981; 3: 74-79.

Ziv G. Drug selection and use in mastitis: systemic vs local therapy. J Am Vet Med Assoc 1980a; 176: 1109-1115.

Ziv G. Experiments with spiramycin/neomycin intramammary dry-period treatment. 9e Congres International sur les Maladies du Betail, Paris. Rapports & Resumes. 1976; Tome 2. 1065-1072.

Ziv G. Good practice in the treatment of mastitis: selecting the ideal. SFB, Paris, 1994; 206-218.

Ziv G. Practical pharmacokinetic aspects of mastitis therapy-1. Parenteral treatment. Vet Med Small Anim Clin 1980b; 64: 277-290.

9. LIST OF PUBLICATIONS OF THE THESIS

Refereed research papers published in scientific journals

In domestic journals, in Hungarian language

Huszenicza Gy., Kulcsár M., Thúróczy J., Jánosi Sz., Kóródi P., Nagy P., Fekete S., Balogh L. A mellékvese-kéreg mûködésének néhány diagnosztikai és kórtani vonatkozása szarvasmarhán. Irodalmi áttekintés. Magy. Áo. Lapja, 1997; 119: 143-147.

Huszenicza Gy., Jánosi Sz., Kulcsár M., Kóródi P., Kátai L. Egyes endotoxin mediálta betegségek endokriológiai és szaporodásbiológiai következményei gazdasági haszonállatokban. Irodalmi áttekintés. Magy. Áo. Lapja, 1998; 120: 329-335.

Jánosi Sz., Laukó T., Huszenicza Gy., Rátz F., Szigeti G., Kerényi J., Kulcsár M., Katona F. A szarvasmarha Prototheca zopfii alga által előidézett tőgygyulladása. Irodalmi összefoglaló. Magy. Áo. Lapja, 1999; 121: 401-406.

Jánosi Sz., Rátz F., Laukó T., Szigeti G., Kerényi J., Tenk M., Katona F., Kulcsár M., Huszenicza Gy. .A szarvasmarha Prototheca zopfii alga okozta tőgygyulladása: a megbetegedés első magyarországi megállapítása. Magy. Áo. Lapja, 2000; 122: 7-14.

Jánosi Sz., Huszenicza Gy. A tejelő tehenek szárazraállítási terápiájának szerepe a tőgygyulladás elleni védekezésben. Magy. Áo. Lapja, 2001; 123: 411-416.

Jánosi Sz., Huszenicza A., Horváth T., Gémes F., Kulcsár M., Huszenicza Gy. Staphylococcus aureus tőgygyulladásban szenvedő tejelő tehenek spiramicinnel végzett szárazraállítási terápiája. Előtanulmányok. Magy. Áo. Lapja, 2001; 123: 417-420.

Baltay Zs., Jánosi Sz. A tőgy antimikrobiális védekezőrendszerének elemei. Állattenyésztés és takarmányozás. 2002; 51: 2. 105-115.

Jánosi Sz., Kacskovics I., Veresegyházy T., Huszenicza Gy. Tőgygyulladásra hajlamosító anyagcsere-rendellenességek és hiányállapotok szarvasmarhán. Irodalmi áttekintés. Magy. Áo. Lapja, 2002. submitted for publication

In domestic journals, in English

Huszenicza Gy., Kégl T., Kulcsár M., Oláh B., Gacs M., Oppel K., Stollár Zs., Jonsson P., Jánosi Sz. Diagnostic value of certain mastitis markers in following up the clinical and bacteriological changes in pharmacotherapeutic studies. Acta Vet. Hung., 1997; 45: 409-416.

Jánosi Sz., Huszenicza Gy., Kulcsár M., Kóródy P. Endocrine and reproductive consequences of certain endotoxin-mediated diseases in farm mammals. A review. Acta Vet. Hung., 1998; 46: 71-84.

Jánosi Sz., Huszenicza A., Horváth T., Gémes F., Kulcsár M., Huszenicza Gy. Bacteriological recovery after intramuscular or intracisternal spiramycin-based drying-off therapy. Acta Vet. Hung. 2001; 49: 155-162.

In (national or international) foreign journals, in Englishs

Huszenicza Gy., Jánosi Sz., Kulcsár M., Kóródi P., Dieleman S. J., Bartyik J., Ribiczei-Szabó P. Gram negative mastitis in early lactation may interfere with ovarian and certain endocrine functions and metabolism in dairy cows. Reprod. Dom. Anim., 1998; 33: 147-153.

Jánosi Sz., Huszenicza Gy., Kulcsár M., Kóródi P., S. J. Dieleman, Ribiczei-Szabó B., J. A. Nikolic, Bartyik J., Rudas P. The Gram-negative mastitis in early lactation may interfere with IGF-1 and insulin levels, thyroid and adrenal functions and metabolism in dairy cows. Biotechnology, Agronomy, Society and Environment, 1998; 2:. 14.

Jánosi Sz., Rátz F., Szigeti G., Kulcsár M., Kerényi J., Laukó T. Katona F., Huszenicza Gy. Review of the microbiological, pathological and clinical aspects of bovine mastitis caused by the alga Prototheca zopfii. Vet. Quart., 2001; 23: 58-61.

Jánosi Sz., Szigeti G., Rátz F., Laukó T., Kerényi J., Tenk M., Katona F., Huszenicza A., Kulcsár M., Huszenicza Gy. Prototheca zopfii mastitis in dairy cows under continental climatic conditions. Vet. Quart., 2001; 23: 80-83.

Jánosi Sz., Huszenicza Gy. The use of the dry cow therapy in the control of bovine mastitis: A review. Vet. Med. Czech. 2001; 46: 55-60.

Kulcsár M., Jánosi Sz., Fébel H., Kátai L., Novotny-Dankó G., Kiss G., Proháczik A., Gáspárdi A., Szűcs E., Rudas P., Huszenicza Gy. Some diagnostic and pathological aspects of adrenocortical function in cattle. Review of Literature. Vet. Med. Czech. accepted for publication

Nikolić J.A., Kulcsár M., Kátai L., Nedić O., Jánosi Sz. and Huszenicza Gy. .Periparturient endocrine and metabolic changes in healthy cows and in those affected by various forms of mastitis. J. Vet. Med B. submitted for publication

Other refereed papers published in edited non-periodicals

Jánosi Sz., Huszenicza Gy., Kulcsár M., Dieleman S. J., Bartyik J., Ribiczei-Szabó P., Kóródi, P., Rudas P. Hyperketonaemia may predispose for early puerperal mastitis, but mastitis failes to cause long-lasting endocrine and metabolic alterations in herds producing low cell count milk. In: Wensing, Th. (ed.): Production diseases in farm animals. Wageningen Pers, Wageningen, The Netherlands, 1999, p. 314.

Jánosi Sz., Laukó T., Huszenicza Gy., Szigeti G. Long-lasting subclinical mastitis caused by Zygosaccharomyces yeasts in two dairy herds.In: Wensing, Th. (ed.): Production diseases in farm animals. Wageningen Pers, Wageningen, The Netherlands, 1999, p. 367.

Congress papers published in proceeding books

Jánosi Sz., Laukó T., Huszenicza Gy., Szigeti,G. Long-lasting subclinical mastitis caused by *Zygosaccharomyces* yeasts in two dairy herds. Proc. of the Middle-European Buiatrics Congress (21-23 May, 1998, Siófok, Hungary), pp. 149-152.

Jánosi Sz., Huszenicza Gy., Kulcsár M., Kóródi P., Dieleman S., J., Bartyik J., Ribiczei-Szabó P., Rudas P. Interrelations of Gram-negative mastitis, ovarian function and certain metabolic disorders in postpartum dairy cows producing low somatic cell count milk. Proc. of the Middle-European Buiatrics Congress (21-23 May, 1998, Siófok, Hungary), p. 160-164.

Jánosi Sz., LaukóT., Huszenicza Gy., Szigeti G. Long-lasting subclinical mastitis caused by *Zygosaccaromyces* yeasts in two dairy herds. Proc. of the 10th Internat. Conf. on Production Diseases in Farm Animals. (24-28 Aug., 1998, Faculty of Veterinary Medicine, Utrecht, The Netherlands), p.189.

Jánosi Sz., Kerényi J., Huszenicza Gy., Rátz F., Szigeti G., Katona F., Kulcsár M. .Bovine mastitis caused by *Prototheca zopfii*, a colorless alga: first report in Hungary. Proc. of the 1st Middle-European Buiatrics Congress (27-29 May, 1999, Balatonfüred, Hungary), p. 238-240.

Jánosi Sz., Huszenicza A., Horvath T., Gémes F., Kulcsar M., Huszenicza Gy. Bacteriological cure rates after an intramuscular or intracysternal Spiramycin based drying off therapy in *Staphylococcus aureus* infected dairy cows: experiences of a pilot study. .Proc. of 2nd Middle-European Congress for Buiatrics (The High Tatras, Slovakia, 11-13 May, 2000), p. 252-4255.

Jánosi Sz., Huszenicza Gy., Kulcsár M., Dieleman S. J., Bartyik J., Ribiczei-Szabó P., Kóródi P., Rudas P. Hyperketonaemia may predispose for early puerperal mastitis, but mastitis failes to cause long-lasting endocrine and metabolic alterations in herds producing low cell count milk. Proc. of the 10th Internat. Conf. on Production Diseases in Farm Animals (24-28 Aug., 1998, Faculty of Veterinary Medicine, Utrecht, The Netherlands), p. 132.

Further full-text professional papers published in non-scientific journals

Jánosi Sz., Rátz, F., Szigeti, G., Kulcsár, M., Kerényi, J., Katona, F., Huszenicza, Gy. Bovine mastitis caused by the alga Prototheca zopfii: Hungarian experiences. Hung. Agricult. Research, 2000; No. 4: 15-17.

10. ACKNOWLEDGEMENT

I would like to express my honest gratitude to Professor Gyula Huszenicza for his support and intensive efforts in supervising my scientific work.

I would like to express my sincere thanks to Dr. Lajos Tekes, the director of Central Veterinary Institute, who has provided the possibility to carry out this work.

I would also like to express my special thanks to the staff of the Endocrine Laboratory of the Department of Obstetrics and Reproduction, Faculty of Veterinary Science, to Dr. Margit Kulcsár, Kiss Istvánné, and Simonné Czigány Ibolya for their valuable and precise work in hormone analyses.

I will never forget the cooperation with my colleagues at the Central Veterinary Institute, the technical assistance of Sára Schubert and Zsuzsanna Török in the laboratory, the help of Dr. Ferenc Rátz in histopathological examinations, and the assistance of Dr. Gábor Majoros in making microphotos.

I am very grateful to Professor Satu Pyörälä (University of Helsinki, Faculty of Veterinary Medicine) and Dr. Tuula Honkanen-Buzalski (National Veterinary and Food Research Institute, Helsinki) for introducing me into the praxis of bovine mastitis.

Last but not least, I would like to thank to my family for all their tolerance and support. I am grateful my wife, Sarolta, for her generosity and to our children, Áron, Eszter and Nóra, who were very patient in waiting the end of my studies.